GENETIC AND MOLECULAR MECHANISMS OF MONOGENIC EPILEPSIES

Patrick Cossette
Department of Human Genetics
McGill University, Montreal

August 2007

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Ph.D

Your file Votre référence
ISBN: 978-0-494-50802-2
Our file Notre référence
ISBN: 978-0-494-50802-2

À la mémoire du docteur Guy Geoffroy

"Reports in support of hereditary factors in epilepsy date back at least to biblical times... The findings of the earlier as well as the more recent investigators suggest that, if hereditary factors underly some of the epilepsies, this is most likely to be demonstrated when the proband has no apparent extrinsic cause for his epilepsy. And, to hide our ignorance here, we say that the patient has idiopathic epilepsy."
KATHERINE AND JULIUS METRAKOS
MONTREAL, 1961.

TABLE OF CONTENTS

ABSTRACT

Epilepsy is a common and heterogeneous neurological disorder affecting aproximately 1.0% of the general population. In up to 65% of cases, the etiology could not be demonstrated (*idiopathic*). Based on familial aggregation and twin studies, genetic factors are believed to play an important role in idiopathic epilepsies. Recently, familial forms of epilepsies have been identified, and mutations in specific genes predisposing to these rare forms of monogenic epilepsies were uncovered. In this thesis, identification of mutations in three different genes is described, including a D188V mutation in the α1 subunit of the voltage-gated sodium channel (*SCN1A*) associated with generalized epilepsy with febrile seizure plus; an A322D mutation in the α1 subunit of the $GABA_A$ receptor in an autosomal dominant form of juvenile myoclonic epilepsy; and a Q555X mutation in synapsin 1 (*SYN1*) in a recessive X-linked temporal lobe epilepsy. Preliminary functional studies on each of these mutation suggested altered function of the mutated genes, suggesting that these mutations are indeed pathogenic. Also, detailed clinical characterization of the $SYN1_{Q555X}$ mutation carriers revealed that affected males and females respectively exhibit developmental language disorder and dyslexia. Considering that individuals with mutated *SCN1A* may also exhibit mental retardation, these data suggest that mutations in epilepsy genes may also predispose to impaired cognition. The identification of such molecular mechanisms underlying monogenic epilepsies should eventually help to improve the treatment of this chronic and debilitating disorder, and will provide food for a revised, molecular-based, classification of the epileptic syndromes.

RÉSUMÉ

L'épilepsie est une maladie neurologique fréquente et hétérogène qui touche environ 1.0 % de la population générale. L'étiologie demeure indéterminée dans plus de 65% des cas. Les études d'agrégation familiale et de jumeaux identiques suggèrent que les facteurs génétiques contribuent de façon importante aux épilepsies idiopathiques. Des formes familiales d'épilepsie ont été récemment indentifiées et des mutations dans des gènes spécifiques, prédisposant à ces formes rares d'épilepsie monogénique, ont été retrouvées. Dans cette thèse, l'identification de mutations dans trois gènes est décrite, incluant une mutation D188V dans la sous-unité α1 du canal sodique voltage-dépendant (*SCN1A*), associé à l'épilepsie généralisée avec convulsions fébriles plus; une mutation A322D dans la sous-unité α1 du récepteur $GABA_A$ (*GABRA1*), associée à une forme autosomique dominante d'épilepsie juvénile myoclonique; et une mutation Q555X dans la synapsine 1 (*SYN1*), associée à une forme d'épilepsie temporale avec transmission récessive liée à l'X. Nos études préliminaires montrent que la fonction normale de ces gènes est altérée pour chacune de ces mutations, suggérant qu'elles sont effectivement pathogènes. Aussi, l'analyse des porteurs de la mutation $SYN1_{Q555X}$ mutation a révélé que celle-ci est associée avec un trouble du développement du langage chez les hommes et la dyslexie chez les femmes. Considérant que les individus avec des mutations dans *SCN1A* peuvent présenter un retard intellectuel, ces données suggèrent que les gènes prédisposant à l'épilepsie peuvent également prédisposer à une perturbation de la cognition. L'identification des mécanismes moléculaires sous-jacents aux épilepsies monogéniques pourrait faciliter le dévelopment de nouveaux traitements pour cette maladie et permettre l'élaboration d'une version révisée de la classification des syndromes épileptiques.

ACKNOWLEDGEMENTS

I first want to express my gratitude to my supervisor Guy Rouleau. As an outstanding researcher, he is totally dedicated to his work, passionated by his laboratory, and fascinated by science. Throughout my training in his laboratory he was a source of inspiration, a gifted teacher, and a generous mentor. I am indebted for having him as a guide not only during my PhD, but also in my first steps as an independent investigator. Guy, many thanks again for everything.

I have a special thank for the families who participated in our study. Whithout them, no genetic project can be achieved. I also want to thank all the neurologists who brought them to our attention, particularly Drs Guy Geoffroy, Dang Khoa Nguyen, Michel Vanasse, Lionel Carmant, and Anne Lortie.

I am also very grateful to Bernard Brais, Jean-Marc Saint-Hilaire, Pierre Duquette and Normand Giard, who encouraged me from the beginning to pursue my research training. Their enthusiasm and support towards my work was very much appreciated. They all had a major influence in the orientation of my carreer in epilepsy and genetics.

I also want to thank Robert Dunn, my co-supervisor, who always kept his door and mind opened for my questions. He was also very helpful, notably by catalysing collaboration between the basic scientists and our team of clinical geneticists.

Any research project is a team effort that requires the participation of many talented players. With this in mind, I want to thank and acknowledge the contribution of Daniel Rochefort, Kateri Brisebois, Judith St-Onge, and Line Lapointe for their technical assistance; Ron Lafrenière and Claude Marineau for their judicious advice; Dominique

Verlaan, André Toulouse, Collette Hand and François Gros-Louis, my labmates, for their ideas and help in data analysis; Sylvie Toupin and Micheline Gravel for their help in recruiting and collecting families; David Ragsdale, Andrew Loukas, and Eric Harvey for the electrophysiological experiments on sodium channels; Lidong Liu and Yu-Tian Wang for the electrophysiological experiments on $GABA_A$ receptors; Joe McDearmid and Pierre Drapeau for the zebrafish experiments; Genevève Sénéchal, Isabelle Rouleau, Karine Marcotte, and Ana Inès Ansaldo for assessment of neuropsychological and language abilities.

Many thanks to Catherine Boyer for her help in formatting the references, and Dominique Verlaan for proof reading of the thesis. I am also grateful to the Canadian Institutes for Health Research for their financial support.

My final thanks go to my family. First to my parents, Robert and Charlotte, who gave me the most important things I needed: their love, their attention, a lot of their time, and their unconditional support for my numerous projects; to my wife Annie, who accompanied and supported me from the beginning, throughout this long journey of medical school, specialisation in clinical neurology and a PhD in Human Genetics; and finally to my two sons, Julien and Laurent, who shared their Dad with his demanding carreer.

CONTRIBUTIONS OF AUTHORS

Chapter 2

Cossette P, Loukas A, Lafrenière RG, Harvey E, Rochefort D, Ragsdale DS, Dunn R, and Rouleau GA. Functional characterization of the D188V mutation in *SCN1A* causing generalized epilepsy with febrile seizures (GEFS). *Epilepsy Research* 2003; 53: 107-17.

Patrick Cossette performed the experiments, except for electrophysiology, and wrote the paper;

Andrew Loukas performed the electrophysiology experiments, participated to the cloning of the D188V mutation and contributed to the writing of the paper;

Ronald G. Lafrenière contributed to the design of the study and to the writing of the paper;

Daniel Rochefort provided technical assistance;

Eric Harvey-Girard contributed to the electrophysiology experiments;

David S. Ragsdale supervised the electrophysiology experiments and contributed to the writing of the paper;

Robert J. Dunn was the thesis co-supervisor and contributed to the writing of the paper;

Guy A. Rouleau was the thesis supervisor and contributed to the writing of the paper.

Chapter 3

Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, and Rouleau GA. Mutation of *GABRA1* in an autosomal dominant form of Juvenile Myoclonic Epilepsy. *Nature Genetics* 2002; 31: 184-189.

Patrick Cossette collected and examined the large family with juvenile myoclonic epilepsy, performed the experiments and analysis (except for electrophysiology), and wrote the paper;

Lidong Liu performed the electrophysiology experiments, and contributed to the writing of the paper;

Haiheng Dong contributed to the electrophysiology experiments;

Katéri Brisebois and *Andrei Verner* provided technical assistance;

Anne Lortie, *Michel Vanasse*, *Jean-Marc Saint-Hilaire*, and *Lionel Carmant* provided patient samples and clinical information;

Wei-Yang Lu and *Yu Tian Wang* supervised the electrophysiology experiments and contributed to the writing of the paper;

Guy A. Rouleau was the thesis supervisor and contributed to the writing of the paper.

Chapter 4

Cossette P, Patry L, McDearmid JR, Ansaldo AI, Rouleau I, Sénéchal G, Marcotte K, Mottron L, Lapointe L, St-Onge J, Verlaan DJ, Gros-Louis F, Lortie A, Carmant L, Raskind WH, Nguyen DK, Drapeau P, and Rouleau GA. Synapsin 1 truncation is associated with developmental dyslexia, language impairment and epilepsy. Submitted.

Patrick Cossette collected and examined the large family with temporal lobe epilepsy and dyslexia, participated in the characterization of language profiles of mutation carriers, planned and supervised the experiments, performed most of the analysis (except for electrophysiology), and wrote the paper;

Lysanne Patry performed many experiments and genetic analyses, and contributed to the writing of the paper;

Jonathan R. McDearmid performed the *in vivo* characterization of the mutation in zebrafish, including electrophysiology experiments, and contributed to the writing of the paper;

Geneviève Sénéchal, *Karine Marcotte*, and *Isabelle Rouleau* assessed the language and cognitive performances of mutation carriers;

Ana Ines Ansaldo supervised the characterization of language profiles of mutation carriers, and contributed to the writing of the paper;

Laurent Mottron characterized the psychiatric profiles of mutation carriers, and contributed to the writing of the paper;

Line Lapointe and *Judith St-Onge* provided technical assistance;

Dominique Verlaan contributed to the genetic analyses and to the design of the study;

François Gros-Louis contributed to the *in vivo* characterization of the mutation in zebrafish;

Anne Lortie, *Lionel Carmant*, *Wendy H. Raskind*, *Dang Khoa Nguyen* provided patient samples and clinical information;

Pierre Drapeau participated in the design of the study, and contributed to the writing of the paper;

Guy A Rouleau was the thesis supervisor and contributed to the writing of the paper.

CLAIMS FOR ORIGINALITY

The overall aim of this Ph.D work is to identify genes and molecular mechanisms conferring susceptibility to epilepsy, by focusing on familial forms of idiopathic epilepsies. With these respects, the original findings presented in this thesis are:

1) in **chapter 2**, the identification a D188V mutation in the α1 subunit of the voltage-gated sodium channel (*SCN1A*) in a large pedigree is described, the original family from which the syndrome of Generalized Epilepsy with Febrile Seizures plus (GEFS+) has been identified (Scheffer and Berkovic 1997). By recording sodium channel currents *in vitro*, we found that recombinant *SCN1A* channels harbouring the D188V mutation were more resistant to the decline in amplitude that is normally observed over the course of high frequency pulse trains. This change in channel function is compatible with an increase in membrane excitability, such as during sustained and uncontrolled neuronal discharges;

2) in **chapter 3**, the identification of the first gene predisposing to juvenile myoclonic epilepsy (JME), one of the most common epilepsy syndrome, is described. This finding started with the identification in my epilepsy clinic of a large family segregating JME with autosomal dominant inheritance, in which we have found a missense mutation in the α1 subunit of the $GABA_A$ receptor (*GABRA1*). By performing *in vitro* recording of GABA-evoked currents, we showed that the $GABRA1_{A322D}$ mutation is associated with a loss-of-function of this ligand-gated chloride channel.

3) in **chapter 4**, the identification of a large family with recessive X-linked temporal lobe epilepsy caused by a Q555X mutation in synapsin 1 (*SYN1*) is described. By knocking-down *Syn1* in zebrafish, we showed that the $SYN1_{Q555X}$ mutation is associated with impaired synaptic transmission *in vivo*;

4) in **chapters 2 and 3** of this thesis, examples where a mutation in the same gene is associated with different clinical phenotypes are provided, supporting the hypothesis of a biologic continuum among generalized epilepsy syndromes. The variety of phenotypes associated with mutations in a single epilepsy gene may ultimately lead to a revised, molecular-based, classification of epilepsy syndromes.

5) in **chapter 4**, we show that, in addition to epilepsy, males carrying $SYN1_{Q555X}$ mutations exhibit language disorder, whereas female exhibit dyslexia and language disorder as a dominant X-linked trait. This observation suggests that *SYN1* may also predispose towards a rare form of language disorder with Mendelian inheritance.

ABBREVIATIONS

ADNFLE	autosomal dominant nocturnal frontal lobe epilepsy
ADPEAF	autosomal dominant partial epilepsy with auditory features
ARFGAP	ADP-ribosylation factor GTPase activating protein
BECTS	benign childhood epilepsy with centrotemporal spikes
BFNIC	benign familial neonatal-infantile convulsions
BNC	bening neonatal convulsions
BNFC	benign neonatal familial convulsions
CACN	voltage-gated calcium channel
CAE	childhood absence epilepsy
CEOP	childhood epilepsy with occipital paroxyms
CHRN	nicotinic acetylcholine receptor
CLCN	voltage-gated chloride channel
CPS	complex partial seizures
EA	episodic ataxia
EEG	electroencephalogram
EFHC	EF-hand calcium-binding motif
EGMA	epilepsy with grand mal seizures on awakening
EVLD	epilepsy with variable learning disabilities
FEB	familial febrile seizure
FHM	familial hemiplegic migraine
FICPC	familial infantile convulsions and paroxysmal choreoathetosis
FKC	paroxysmal kinesigenic choreoathetosis
FS	febrile seizures
GABA	gamma-aminobutyric acid
GABRA	alpha subunit, gamma-aminobutyric acid receptor
GABRD	delta subunit, gamma-aminobutyric acid receptor
GABRG	gamma subunit, gamma-aminobutyric acid receptor
GEFS+	generalized epilepsy with febrile seizure plus
GEPD	generalized epilepsy with paroxysmal dyskinesia
GSW	generalized spike waves
GTC	generalized tonic-clonic
IGE	idiopathic generalized epilepsy
JAE	juvenile absence epilepsy
JME	juvenile myoclonic epilepsy
KCN	voltage-gated potassium channel
LGI1	leucin-rich glioma-inactivated
MRI	magnetic resonance imaging
PEPS	partial epilepsy with pericentral spikes
REPED	rollandic epilepsy with paroxysmal exercise-induced dystonia
SCN	voltage-gated sodium channel
SYN	synapsin
TLE	temporal lobe epilepsy

CHAPTER 1: GENERAL INTRODUCTION

HISTORICAL PERSPECTIVES

Epilepsy is the most common neurological disease after stroke, affecting approximately 1.0% of the general population. The disease has been recognized and described by physicians since Ancien times. The earliest description of epileptic seizures was found in the *Sakikku*, a Babylonian cuneiform medical diagnostic text compiled between 1067 BC and 1046 BC (Wilson and Reynolds 1990; Eadie and Bladin 2001). In the *Sakikku*, what appear to be epileptic episodes were called *antasubba* (in Sumerian) or *miqtu* (Akkadian term) which means "the falling disease" (Wilson and Reynolds 1990). The word *epilepsy* and its Latin equivalent *epilepsia* are derived from the Greek επιλημψιξ for *to seize* or *take hold of*, indicating that the individual having a seizure is *possessed* or *out of control* (Temkin 1971; Trescher and Lesser 1996; Eadie and Bladin 2001). Mainly because of its dramatic clinical manifestations, the disease has always provoked astonishment if not fear, providing food for sustained misconceptions about the origin of the disease (Goldensohn 1997). Until relatively recent years, the nature of epilepsy was commonly understood in the general community as an outcome of the activity of supernatural powers, of different kinds. Remnants of such beliefs remain even in advanced societies (Eadie and Bladin 2001). As an example *Grand Mal* and *Petit Mal* seizures are still largely used in the medical literature, especially in continental Europe, to designate generalized tonic-clonic and absence seizures respectively (ILEA 1989; Loiseau et al. 2002; Wolf 2002).

Contemporary understanding of the disease has been largely influenced by the work of John Hughlings Jackson (Jackson 1870; Jackson 1887; Trescher and Lesser 1996;

Goldensohn 1997; Eadie and Bladin 2001) and Wilder Penfield (Pendfield and Jasper 1954; Goldensohn 1997; Eadie and Bladin 2001). These authors notably demonstrated that epilepsy can be caused by various lesions in the brain, and that surgical excision of these *discharging lesions* may cure the disease. However, it is now well established that, in the majority of the individuals with epilepsy, the cause cannot be identified, despite a thorough evaluation. So far, in these *idiopathic* epilepsies, sophisticated magnetic resonance imaging (MRI), as well as neuropathological studies repetitively failed to demonstrate the causative lesion in the brain. In these cases, genetic factors have been suspected to play an important role in the etiology of the disease. However, it is only recently that specific genes, predisposing to rare inherited forms of epilepsy, have been identified. *In this thesis, this recent literature will be reviewed, and a strategy will be elaborated in order to identify additional genes and molecular mechanisms underlying familial forms of idiopathic epilepsies.*

DEFINITION AND CLASSIFICATION

Epileptic seizures

The definition of epilepsy varies significantly from one author to the other, and none of these definitions have been generally accepted in the neurological literature. Over the centuries, the word has been used almost interchangeably for both epileptic episodes (the epileptic seizure), and for the abnormal state of the brain underlying these episodes (epilepsy as a neurological disorder). John Hughlings Jackson in 1870 provided the most accepted definition of epileptic seizure, that he considered as "*an occasional, an excessive, and a disorderly discharge of grey matter*" (Jackson 1870). More recently,

Aicardi proposed that "*an epileptic seizure results in a paroxysmal disorganization of one or several brain functions*" (Aicardi 1986). These two definitions emphasized either on the physiological or the clinical component of the epileptic seizure. Contemporary authors will eventually take into consideration both aspects of these definitions, considering that epileptic seizure is caused by "*a temporary physiological dysfunction of the brain, caused by a self-limited, abnormal, and hypersynchronous electrical discharge of a large group of cortical neurons*" (Martin 1991; Pedley et al. 1995; Fisher et al. 2005).

According to the International Classification of Epileptic Seizures (ILEA 1981), a seizure is considered *partial* when the epileptic discharge is initially limited to part of one brain hemisphere, or *generalized* when it involves both cerebral hemispheres from the onset. The partial seizures are sub-divided into *simple* partial seizure when full consciousness is preserved and *complex* partial seizure when consciousness is impaired or lost during the episode. Any partial seizure may become *secondary generalized* when the seizures eventually spread to both hemispheres, evolving to a generalized tonic-clonic seizure.

The clinical manifestations of any partial seizure will depend on several factors, including: 1) whether most or only part of the cerebral cortex is involved at the beginning; 2) the function of the cortical areas involved by the epileptic discharges (e.g. sensory, motor, visual, limbic) ; 3) the subsequent pattern of spread of the electrical discharge within the brain (Pedley et al. 1995). In addition to partial seizures, the International Classification of Epileptic Seizures recognizes four main types of generalized seizures (ILEA 1981; Blume et al. 2001):

1) *absence* seizure, consisting in a sudden onset, interruption of ongoing activities, a blank stare, often with a brief upward rotation of the eyes, that is usually lasting a few seconds. The clinical manifestations are associated with typical generalized spike-and-wave discharges at the frequency of 3 Hz on electroencephalographic (EEG) recording;

2) *myoclonic* seizures, consisting in a sudden, brief involuntary contraction(s) of muscle(s), of variable topography (e.g. bilateral, unilateral, axial, proximal limb, distal) generally involving the upper limbs and upper half of the body. Repetitive bilateral myoclonic jerks may eventually progress to a generalized tonic-clonic seizure;

3) *atonic* seizures, resulting from a sudden loss or diminution of muscle tone, involving head, trunk, jaw, or limb musculature, which may lead to unexpected fall;

4) *tonic*, *clonic* and *tonic-clonic* seizures, respectively consisting in sustained (tonic), repetitive and rhythmic (clonic) muscle contraction, or combination of both (tonic-clonic).

Except for minor myoclonic jerks, consciousness is usually lost from the beginning in all types of generalized seizure, and these seizures are not preceded by any warning (*aura*).

Epileptic syndromes

Epileptic seizure usually indicates a disturbance in the brain function, which may occur because of a non-specific acute medical or neurological illness. As an example, various psychotropic drug intoxication or withdrawal, as well as acute traumatic head injury may provoke seizure in any individual (Loiseau 1997). Sometimes, a single seizure may arise for unknown reasons (*unprovoked*). In these examples, when the seizures do not persist beyond the acute neurological illness, or when there is no

recurrence after a first unprovoked seizure, the individual is usually not considered epileptic. Epilepsy is rather used to designate a pathological state characterized by recurrent epileptic seizures (Pedley 1997; Fisher et al. 2005; Engel 2006). However, epileptic individuals may exhibit a wide range of clinical presentation, not only in terms of seizure manifestations, but also exhibit variable age of onset, etiology, prognosis, etc. Therefore, epilepsy is clearly not a single condition, but rather a diverse family of disorders, called *the epilepsies*, having in common an abnormally increased predisposition to seizures (Fisher et al. 2005).

Despite high heterogeneity among the various epilepsies, clusters of clinical signs and symptoms have been frequently observed among epileptic individuals, allowing physicians to identify unique clinical entities, called *epileptic syndromes*. The International League Against Epilepsy (ILEA) has started in 1985 to make an inventory of epileptic syndromes widely accepted in the epilepsy community, a process that is still underway (ILEA 1989; Engel 1998; Engel 2001; Engel 2006). The International Classification of the Epilepsies and Epileptic Syndromes rests on two criteria: 1) *seizure type* which may be generalized or partial; and 2) *etiology*, which may be idiopathic, cryptogenic and symptomatic (ILEA 1989) (Table 1 and 2). Additional criteria include: age of onset, evolution of the syndrome, associated interictal signs, symptoms and EEG patterns, and genetic basis (ILEA 1989; Engel 2001; Engel 2006)

Symptomatic epilepsies have multiple and heterogeneous causes including brain injury, central nervous system infection, and metabolic disorders (ILEA 1989; Engel 2006). Also, some hereditary disorders may present with epilepsy, along with a diffuse progressive brain dysfunction (eg. progressive myoclonic epilepsies) (Lohi et al. 2006).

These epilepsies will not be considered in this thesis. In turn, the majority individuals with epilepsy (65%) have recurrent unprovoked seizures without detectable structural lesion in the brain, and without showing any neurological abnormality between seizures. For these individuals, no underlying cause could be identified, and they are said to have an *idiopathic* epileptic syndrome (ILEA 1989; Engel 2001; Engel 2006). From the beginning of the International Classification, the term idiopathic epilepsies has been used for those in which "*there is no underlying cause other than a possible hereditary predisposition*" (ILEA 1989; Engel 2001; Engel 2006). There is now increasing evidence that in this latter group, genetic factors are playing an important role. *The overall aim of this Ph.D work is to identify additional genes conferring susceptibility to epilepsy, by focusing on familial forms of idiopathic epilepsies, where the genetic predisposition seems to be most striking.*

Idiopathic generalized epilepsies and syndromes

According to the classification of epileptic syndromes proposed by the International League Against Epilepsy (ILEA), individuals with idiopathic generalized epilepsies (IGE) must present the following features : 1) occurrence of generalized seizures; 2) normal interictal state, including normal neurological examination and normal brain imaging; 3) an EEG showing normal background activity and generalized epileptic discharges consisting in spikes, polyspikes, spike wave, and polyspike wave, usually at the frequency of >3Hz. Based on these clinical criteria, a total of seven specific IGE syndromes have been recognized by the ILEA. These syndromes differ mainly in the age of onset, and are briefly described here.

Benign neonatal familial convulsions (BNFC) is a rare form of epilepsy exhibiting autosomal dominant inheritance, characterized by clonic or apneic seizures occurring in the first three days of life. There are no specific EEG criteria. Psychomotor development is normal, and 14% of these patients later develop epilepsy (ILEA 1989; Plouin and Anderson 2002).

Benign neonatal convulsions (BNC) is a sporadic epilepsy syndrome presenting with frequent clonic or apneic seizures usually occurring on the fifth day of life without known etiology. EEG may show alternating sharp theta waves. As in BNFC, psychomotor development is normal, but there is no recurrence of seizures later in life (ILEA 1989; Plouin and Anderson 2002). Recent video-EEG recordings have shown that individuals with either BNFC or BNC may also have partial seizures (Plouin and Anderson 2002). Based on these findings, several authors questioned the inclusion of these latter syndromes among the idiopathic generalized epilepsies (Engel 2001; Plouin and Anderson 2002).

Benign myoclonic epilepsy in infancy (BMEI) is characterized by brief bursts of generalized myoclonus that occur during the first or second year of life. Familial history of epilepsy is common. EEG recordings show generalized spike-waves typically during early stages of sleep. Generalized tonic-clonic seizures may occur during adolescence. The intellectual development is usually normal (ILEA 1989).

Childhood absence epilepsy (CAE) occurs in children between the ages of 2 to 8 years old. It is more frequent in girls than in boys (approximately 2:1) and is characterized by very frequent absence seizures (up to hundred episodes per day) of brief duration (5 to 20 seconds). EEG recordings show bilateral and synchronous generalized spike-and-wave

epileptic discharges (GSW), usually at the frequency of 3Hz (ILEA 1989; Loiseau et al. 2002). Persistence of absences during adulthood is uncommon, but approximately 40% of individuals with CAE eventually develop generalized tonic-clonic (GTC) seizures during adolescence, that may persist into adult life (Loiseau et al. 2002). CAE represents approximately 29% of all IGE (Desguerre et al. 1994; Roger et al. 1994).

In *juvenile absence epilepsy* (JAE), the absence seizures are the same as in CAE, except for the age of onset which is between the ages of 8 to 16 years old. Seizure frequency is generally lower than in CAE, and sex distribution is equal. Association with GTC seizures is frequent, and these individuals may have myoclonic seizures (ILEA 1989; Wolf and Inoue 2002). EEG also shows GSW, often at a frequency of 3Hz or faster. JAE represents approximately 11% of all IGE (Roger et al. 1994; Wolf and Inoue 2002).

Juvenile myoclonic epilepsy generally appears around puberty, but may start from age of 8 to 20 years old. This syndrome is characterized by myoclonic jerks that predominate in the arms. These myoclonic seizures are frequently associated with GTC seizures and, less often, with infrequent absences. As for JAE, sex distribution is equal. The seizures usually occur shortly after awakening and are often precipitated by sleep deprivation. EEG shows rapid (>3,5 Hz), often irregular spike-and-wave and poly-spike-and-wave discharges (ILEA 1989; Thomas et al. 2002). Frequently, the GSW discharges are precipitated by photic light stimulation (photosensitivity) (Thomas et al. 2002). This epilepsy generally needs long-term antiepileptic drug treatment. Indeed, withdrawal of antiepileptic drugs is associated with seizure recurrence in up to 90% of cases (Delgado-Escueta and Enrile-Bacsal 1984). JME is a common epileptic syndrome and represents

between 5-10% of all the epilepsies, and aproximately 23% of all the IGE (Roger et al. 1994; Thomas et al. 2002).

Epilepsy with Grand Mal on awakening (EGMA) presents with GTC seizures occurring exclusively or predominantly shortly after awakening. Absences or myoclonic seizures may also occur. The EEG shows one of the IGE pattern described above (ILEA 1989; Wolf 2002). As for JME, this syndrome is frequently associated with photosensitivity (Wolf 2002). EGMA represents aproximately 12% of all IGE (Roger et al. 1994). Among individuals with idiopathic GTC seizures, approximately 33% have GTC seizure on awakening (Wolf 2002).

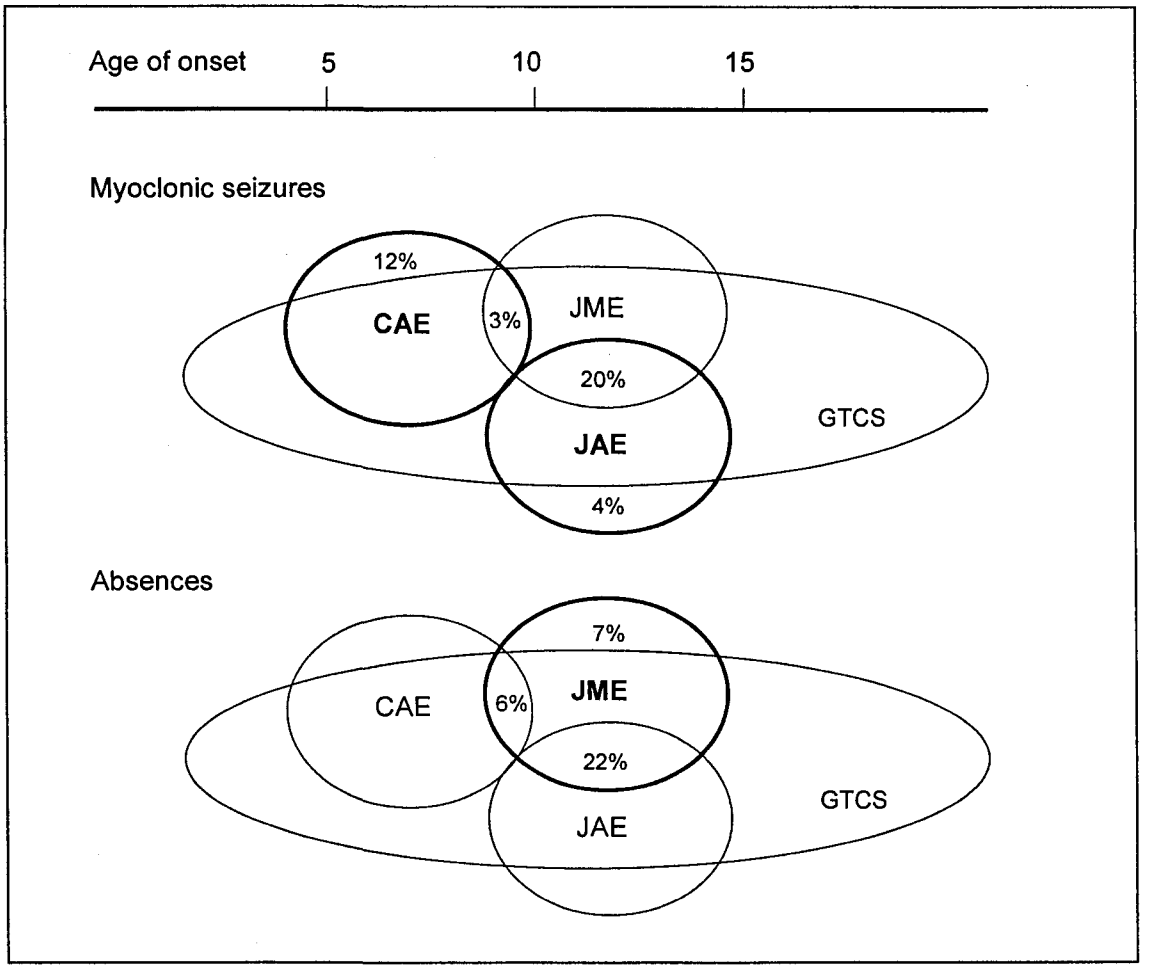
Whereas BNC, BNFC and BMEI are rare epileptic syndromes, childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and epilepsy with Grand Mal on awakening (EGMA) are common epilepsies. These "classical" IGE syndromes represents between 25 to 30% of all the epilepsies (Desguerre et al. 1994; Roger et al. 1994). However, in clinical practice, it is not always easy to diagnose a definite IGE syndrome in a given individual. In particular, significant overlaps exist between the classical IGE syndromes, especially those with onset during adolescence (Andermann and Berkovic 2001; Engel 2001; Thomas et al. 2002; Engel 2006) (Figure 1). As an example, only the quantitative predominance of myoclonic jerks, and the fact that GTC seizures are preceded by a volley of myoclonic jerks, will push the diagnosis towards JME rather than EGMA (Thomas et al. 2002). Based on the overlaps in the clinical manifestations among the classical IGE syndrome, several authors suggested that these epilepsies may be part of a biological continuum, rather than discrete diagnostic entities (Berkovic et al. 1987; Thomas et al. 2002; Wolf 2002). *In chapters 2*

and 3 of this thesis, I will provide examples where mutation in the same gene is associated with different clinical phenotypes, supporting the hypothesis of a biologic continuum among generalized epilepsy syndromes.

Table 1. Idiopathic generalized epilepsy syndromes

- Benign neonatal familial convulsions
- Benign neonatal convulsions
- Benign myoclonic epilepsy in infancy
- Childhood absence epilepsy
- Juvenile absence epilepsy (pyknolepsy)
- Juvenile myoclonic epilepsy (impulsive petit mal)
- Epilepsy with grand mal seizures (GTCS) on awakening
- Other generalized idiopathic epilepsies
- Epilepsies with seizures precipitated by specific modes of activation

Figure 1. Overlap between classical idiopathic generalized epilepsy syndromes*



*From Thomas, 2002

Idiopathic partial epilepsies and syndromes

In the original classification of the ILEA, only two idiopathic partial epilepsy syndromes were recognized, which share many common clinical features (ILEA 1989). Both syndromes are age-related, without demonstrable anatomic lesions, and are subject to spontaneous remission. Affected individuals have neither neurological and intellectual

deficit, nor a history of antecedent illness. The seizures are usually brief and rare. The EEG is characterized by normal background activity and localized high-voltage repetitive spikes, that are increased by sleep.

Benign childhood epilepsy with centrotemporal spikes (BECTS) is characterized by simple partial, hemifacial motor seizures frequently associated with somatosensory symptoms, which may evolve into GTC seizure, most of the time during sleep. The onset is usually around 9 years, and remission invariably occurs before the age of 16 years. The EEG is characterized by high-voltage centro-temporal spikes, activated by sleep, which tend to spread or shift from one hemisphere to the other (ILEA 1989; Dalla Bernardina et al. 2002).

Childhood epilepsy with occipital paroxyms (CEOP) is characterized by visual symptoms, often followed by hemiclonic seizures. The seizures may be followed by migrainous headache in 25% of the cases. The EEG is characterized by high-amplitude spike-waves on the occipital and posterior temporal areas of one or both hemispheres (ILEA 1989; Panayiotopoulos 2002).

In both BECTS and CEOP, familial history of epilepsy may be found (ILEA 1989; Dalla Bernardina et al. 2002; Panayiotopoulos 2002). Overall, the pattern of inheritance does not suggest Mendelian heredity. Also, although putative loci have been identified for BECTS (Dalla Bernardina et al. 2002), specific genes predisposing to this epileptic syndrome remain to be identified.

Table 2. Idiopathic partial epilepsy syndromes

- Benign childhood epilepsy with centrotemporal spikes
- Childhood epilepsy with occipital paroxysms

Limitations and evolution of the current classification of epileptic syndromes

Classification of seizures and epileptic syndromes is useful in clinical practice, allowing clinicians to determine a rational plan of investigation, making decisions on when and how long to treat, choosing the appropriate antiepileptic drug, and conducting scientific investigations that require delineation of clinical and EEG phenotypes, including genetic studies (Pedley et al. 1995; Engel 2001). Whereas the classification of epileptic seizures is generally well accepted, the classification of the epileptic syndromes is more controversial, and remains a matter of debate (Pedley et al. 1995; Engel 1998; Engel 2001; Wolf 2003; Engel 2006). Many authors pointed out that the current classification scheme is empiric, with clinical and EEG data emphasized over anatomic, pathologic or specific etiologic information (Pedley et al. 1995; Wolf 2003). On the other hand, this imperfect classification reflects that only a minority of the epilepsies is so far explained by a specific disease or defect. The classification is thus an ongoing process, which continues to evolve. Among these changes, the appellation *cryptogenic* epilepsies, previously used to design an *epileptic syndrome caused by a yet unidentified structural brain lesion*, will probably be abandoned (ILEA 1989; Engel 2001). Finally, based on genetic studies of large kindred segregating specific clustering of epileptic manifestations (see p. 37), additional idiopathic generalized and partial epilepsy syndromes have been recently described (Table 3). These latter syndromes are considered as potentially new categories in future ILEA classification (Engel 2001; Engel 2006).

Table 3. Additional idiopathic epilepsy syndromes (in development)

- Generalized epilepsy with febrile seizures plus
- Autosomal dominant nocturnal frontal lobe epilepsy
- Familial temporal lobe epilepsy
- Famial partial epilepsy with variable foci

COGNITIVE IMPAIRMENTS AND LEARNING DISORDERS ASSOCIATED WITH EPILEPSY

Epilepsy is thus considered to be either a primary disorder of the brain, or the consequence of a wide variety of lesion, both in terms of etiology and localization. Therefore, disturbance of these cortical networks could not only lead to the ictal symptomatology, but also impair high-order cognition, learning processes, behaviour and socialization. Neurological and psychiatric disorders associated with epilepsy have been recognized long time ago by Hughling Jackson who wrote: *"surely the conclusion is irresistible, that 'mental' symptoms from diseases of the hemispheres are fundamentally like hemiplegia, chorea, and convulsions, however specifically different. They must all be due to lack, or disorderly development, of the sensorimotor processes"* (Jackson 1887). Although the majority of authors agree on this concept, the exact mechanisms and neural networks involved in cognition, learning and behavioural disturbance remain largely unknown (Cassidy and Corbett 1997). In the case of epileptic individuals, several, not mutually exclusive, mechanisms may underlie these disorders, including : 1) genetic predisposition to a neurodevelopmental or psychiatric disorder, independent of

the cause of the epileptic syndrome *per se*; 2) brain dysfunction induced by the specific cause of the epileptic syndrome; 3) effects of the seizures themselves; 4) psychological and social environment; 5) antiepileptic drugs and other drug therapy; 6) surgical excision of the epileptic focus (Trimble 1987; Aldenkamp 1997; Cassidy and Corbett 1997; Mandelbaum and Burack 1997; Aldenkamp and Bodde 2005).

Cognitive impairment

A variety of cognitive impairments have been reported in epileptic individuals, particularly in individuals with partial epilepsies intractable to medical treatment (Trimble 1987; Aldenkamp 1997; Mandelbaum and Burack 1997; Aldenkamp and Bodde 2005). Most of the time, these impairments reflects the localization of the causative lesions. In individuals with symptomatic epilepsy, the cognitive deficits may be explained by the anatomical lesion alone (e.g. stroke, tumor). Surgical excision of the epileptic focus within cerebral cortex is another obvious cause for cognitive deficits. By definition, there is no identifiable anatomical lesion in idiopathic epilepsy (ILEA 1989). However, we can conceive that either microscopic (e.g. cortical dysplasia) or 'molecular' lesions (e.g. mutated ion channel) causing idiopathic epilepsies, may also be the substratum for impairment of brain function. Gene defects may thus theoretically cause disturbance in neuronal excitability, neurotransmission or wiring, which may be responsible for cognitive or behavioural disturbance. In addition, impaired metabolism of the surrounding epileptic focus has been well documented in individuals with partial epilepsy, with or without anatomical lesion (Engel et al. 1990; Newton et al. 1995). Therefore, these two mechanisms - functional disturbance caused by the seizures

themselves, and the underlying brain lesion - may lead to specific cognitive impairment in idiopathic epilepsies. The semiology of cognitive deficits associated with various regions of the brain (e.g. frontal, temporal, and parietal lobe syndromes) are well chatacterized entities and will not be described here.

Mental retardation

In addition to specific cognitive impairment, epilepsy may be associated with widespread dysfunction of the brain leading to globally impaired cognitive abilities. The International Classification for the Disease (ICD-10) defined mental retardation as *"a condition of arrested or incomplete development of the mind that is characterized by impairment of skills, manifested during the developmental period, that contribute to the overall level of intelligence, i.e. cognitive, langage, and social abilities"* (ICD-10 1992). Mental retardation is found in up to 23% of epileptic individuals overall (Cassidy and Corbett 1997), but this rate varies greatly according to the epilepsy syndrome (Beaumanoir and Blume 2002; Dravet et al. 2002; Dulac and Tuxhorn 2002; Guerrini et al. 2002). In turn, up to 31% of people with mental retardation are epileptic, and the frequency of epilepsy increases with the severity of mental retardation (Cassidy and Corbett 1997).

By definition, individuals with idiopathic generalized epilepsy have normal intelligence. In contrast, mental retardation is observed in up to 90% of the individuals with so-called *secondary generalized epilepsy*, such as infantile spasms and West syndrome (Dulac and Tuxhorn 2002), Lennox-Gastaut syndrome (Beaumanoir and Blume 2002), severe myoclonic epilepsy in infancy (Dravet et al. 2002), and myoclonic

astatic epilepsy (Guerrini et al. 2002). In these individuals, cognitive impairment (as well as the epileptic seizures) may reflect widespread brain damage associated with various perinatal injuries or infections, neurodegenerative and metabolic disorders, cortical dysplasia and neurocutaneous syndromes. However, in a significant proportion of individuals with the same clinical syndromes, including mental retardation, the etiology could not be identified (Beaumanoir and Blume 2002; Dravet et al. 2002; Dulac and Tuxhorn 2002; Guerrini et al. 2002). These latter epilepsies are said to be *cryptogenic* in the ILEA classification of epileptic syndromes, as they refer to a potentially 'hidden' cause, that could not be identified with the current diagnostic tools, including normal brain imaging (ILEA 1989). Because familial history is rarely reported in these secondary generalized epilepsy syndromes, genetic etiology has generally not been considered. However, this observation is partly biased by the fact that virtually none of the individuals with secondary generalized epilepsy syndromes will have children, because of severely impaired cognition and social skills. Therefore, one could not exclude that spontaneous mutations may be responsible for these epilepsies. Consistent with this hypothesis, the concordance rate for secondary generalized epilepsies is high in identical twins (up to 83%), and virtually absent (0%) in dizygotic twins (Berkovic et al. 1998).

A common feature in these secondary generalized epilepsy syndromes is the occurrence of early-onset, frequent, severe, and intractable seizures. In addition, some regression in the developmental milestones is often observed after the onset of the seizrures. These latter observations led to an abundant literature suggesting that cognitive impairment and decline seen in these individuals may be caused by repetitive seizures, which could in itself cause brain damage (Beaumanoir and Blume 2002; Dravet et al.

2002; Dulac and Tuxhorn 2002; Guerrini et al. 2002), a phenomenon called *epileptic encephalopathy* (Engel 2006). The addition of a heavy polymedication in order to control the seizures may also contribute to cognitive impairments (Mandelbaum and Burack 1997). In comparison to adult epileptics, the combined effects of seizures and the anti-epileptic drugs on the cognitition may be even more critical in the developing brain. Again, an alternative hypothesis would be that secondary generalized epilepsy syndromes may be caused by mutations in genes that may lead to a more striking phenotype, including severe epileptic seizures and impaired cognition.

Neurodevelopmental disorders

At least two other groups of neurodevelopmental disorders have been associated with epilepsy: (1) *autism-spectrum disorder*, which may be associated with malignant forms of epilepsy, such as infantile spasms (Dulac and Tuxhorn 2002), Lennox-Gastaut syndrome (Beaumanoir and Blume 2002), and severe myoclonic epilepsy in infancy (Dravet et al. 2002). In addition, epileptic seizures may be observed in up to one third of autistic individuals, and all types of seizures can be seen in these individuals (Olsson et al. 1988; Rapin 1997);

(2) *language disorders*, including developmental dyslexia are more commonly seen in epileptic individuals (Schachter et al. 1993). Also, Landau-Kleffner is a rare epileptic syndrome characterized by severe temporal lobe epilepsy, rapidly progressive acquired aphasia, and autistic features (Landau and Kleffner 1957; Smith 1997). Although a genetic predisposition have been consistently observed in language disorders and autism (Rapin 1997; Fisher and DeFries 2002), the specific genes underlying these disorders largely remain to be identified. Moreover, the biological mechanisms and neural

networks involved in autism and language disorders remain so far elusive (Habib 2000; Ramus 2001).

BASIC MECHANISMS UNDERLYING PARTIAL EPILEPSIES

Animal model versus Human studies

Because of the heterogeneity of the epileptic syndromes, one can expect that a variety of cellular and molecular mechanisms will be implicated in the pathogenesis of epilepsy in Humans. The neurobiology of epilepsy has been a matter of considerable research activity over the past decades, giving rise to an extensive literature on this topic. A comprehensive review of these data is thus beyond the purpose of this thesis. We will rather present an overview of the prevailing hypothesis regarding the pathophysiology of the various forms of epilepsy. We will mainly emphasize animal models relevant to human phenotypes, and highlight the established molecular mechanisms that could be relevant in the search for genes predisposing to the disease. Overall, these data on the neurobiology of epilepsy are based on:

1) *Human studies*, including empirical response to the various antiepileptic drugs, *in vivo* imaging, neuropathological examination, as well as electrophysiological and biochemical studies of human brain tissue excised from epileptic individuals;

2) *Animal models* which, in principle, are mimicking various forms of epilepsy. Selected models for the disease, their relevance to human epilepsy, the mechanisms underlying the epileptogenesis processes, as well as those underlying the hyperexcitable state will be presented briefly.

Before reviewing these data, one needs to differentiate between the cellular and molecular changes associated with the seizures themselves, from the basic mechanisms leading to the genetic or acquired forms of epilepsy. Indeed, most of these anatomical and physiological modifications documented in the epileptic brain probably reflect the consequence of the seizures, rather than the cause. With these respects, virtually nothing is known on the epileptogenesis process itself, nor on the primary cause of the common epilepsy syndromes (Dichter 1997). Finally, whereas animal models for temporal lobe epilepsy have been the subject of many studies, very little is known on the pathophysiology of other focal epilepsies.

Animal models for temporal lobe epilepsy

Temporal lobe epilepsy is the most common form of partial epilepsy in Humans, and is often associated with neuronal loss and gliosis in specific regions of the hippocampus, including the end-folium of dentate gyrus (granule cells), CA3 and, to a lesser extent, CA1 region (Du et al. 1993; Gloor 1997). The pathophysiological mechanisms underlying this typical neuropathological lesion called hippocampal sclerosis in Humans remain largely unknown. However, hippocampal sclerosis is more likely to occur in individuals who presented status epilepticus or prolonged febrile seizures in early childhood (Jackson et al. 1998; Lewis 1999; Scott et al. 2003). At least three models of temporal lobe epilepsy reproduce hippocampal sclerosis and clinical seizures similar to human disease (Avanzini and al. 1997), including:

1) *Kindling* model: daily stimulation of amygdala in rats could result in the progressive development of convulsive seizures, a phenomenon called *kindling* (Goddard

1967; Goddard et al. 1969). In the first days of this experiment, the initial subconvulsive electrical stimulus only causes a brief after discharge at the tip of the electrode, without clinical seizure. In contrast, the same stimulus applied over the subsequent days progressively increase the length of these after discharges, which ultimately results in intense partial and secondary generalized seizure. Subsequent studies from Goddard's group hightlighted the progressive and permanent nature of kindling (McNamara and Wada 1997).

2) systemic injection of *kainic acid*, which could be either intravenous, intraperitoneal, or intracerebral (Lothman et al. 1981; Turski et al. 1990). This glutamate analog induces severe and prolonged seizure in rats and mice. After a latent period of approximately 30 days, the animal exhibit spontaneous temporal lobe seizures. Kainic acid-induced seizures are triggered by the activation of excitatory amino acid receptors.

3) systemic injection of *pilocarpine*, a cholinergic agent, which also induces a status epilepticus in rats (Cavalheiro et al. 1991). These animals develop spontaneous recurrent seizures after a latent period of 15 days. The administration of pilocarpine and other cholinergic agents have been shown to block the transmembrane current I_M, which facilitate burst discharges in hippocampal pyramidal (Benardo and Prince 1982).

Interestingly, in addition to the striking neuronal loss in the hippocampus, these three models (kindling, kainic acid, and pilocarpine) exhibit a cascade of events that is reminiscent of classical temporal lobe epilepsy in Humans: an initial brain injury associated with prolonged seizure, followed by a latent period, and eventually recurrent spontaneous seizure. However, the mechanisms underlying the transition from a hyper-

excitable state of the brain to spontaneous seizures (epileptogenesis) remains a matter of debate. In these respects, the prevailing, not mutually exclusive, hypotheses are:

1) *alteration in the fundamental excitability of neurons*: abnormal excitability has been identified in neurons in multiple sites of the kindled brain, including dentate granule cells, CA3 and CA1 pyramidal cells of the hippocampus, pyramidal neurons of the pyriform cortex, and neurons in the basolateral nucleus of amygdala (Knowles et al. 1992; Avoli et al. 2005). In particular, neocortical neurons recorded from both rodents and human epileptic brains are characterized by subthreshold responses. These abnormal properties would reflect the presence of fast and persistent Na+ currents, as well as K+ outward currents including a muscarine-sensitive K+ current and a hyperpolarization-activated inward conductance (Halliwell 1986; Lorenzon and Foehring 1992; Vreugdenhil et al. 2004).

2) *development of novel and aberrant synaptic circuitry*: sprouting of the mossy fiber axons of the dentate granule cells of hippocampus has been found in various animal models of temporal lobe epilepsy (Nadler et al. 1980; Sutula et al. 1988), and is associated with immediate early genes expression of neurotrophins and their receptors (Gall and Isackson 1989; Ernfors et al. 1991). It has been shown that these abnormal projections form recurrent excitatory synapses upon themselves or their neighbors, and contribute to the hyperexcitable state of the kindled brain. Sloviter proposed an alternative mechanism by showing a striking loss of the hilar mossy cell in the hippocampus. These neurons normally excite GABA-containing basket interneurons, and mediate feedback inhibition. Therefore, following the loss of these mossy cells, the basket cells are said to become 'dormant' as they become tonically less active, and lose

their abilities to decrease granule cell excitability (Sloviter 1991; Sloviter 1994). Finally, loss of GABAergic interneurons possibly contribute to reduced inhibition in the hippocampus of individuals with mesial TLE (Houser et al. 1990);

3) *change in synaptic efficacy of pre-exisiting connections*: an alternative hypothesis is that the hyperexcitability of the kindled brain is due to enhance function of a subpopulation of glutamatergic synapses using N-methyl-d-aspartate (NMDA) receptors (McNamara and Wada 1997). Increased sensitivity of CA3 pyramidal cells to NMDA-evoked depolarisation has been shown in kindled animals (Martin 1991). Modified function or an increase in the number of NMDA receptors may contribute to these altered synaptic properties (Yeh et al. 1989; Kraus et al. 1994).

Temporal lobe epilepsy: insights from human studies

Overall, most of the mechanistic studies of animal models with partial epilepsy revealed structural and functional modifications similar to what is found in brain tissue excised from epileptic individuals (Avoli et al. 2005). In general, these data correlate well with the mechanism of action of specific anti-epileptic drugs, which may act on sodium channels (phenytoin, carbamazepine, lamotrigine, topiramate), potassium channels (retigabine), GABAergic (benzodiazepine, phenobarbital) and glutamatergic (topiramate) receptors, GABA metabolism (vigabatrin, leviracetam), as well as in the release of excitatory amino acids (gabapentin, lamotrigine) (Kwan et al. 2001). Recently, Becker *et al* analysed gene expression profiles in hippocampus of human operated for MTLE, and compared with rats with temporal lobe epilepsy induced by pilocarpine. These authors found overexpression of genes involved in cell-matrix interactions, cell

growth and differentiation, neuronal signalling, and transcriptional regulation (Becker et al. 2003).

BASIC MECHANISMS UNDERLYING GENERALIZED EPILEPSIES

Finding the generator for generalized spike-wave discharges

Compared to partial epilepsy, the investigation of basic mechanisms underlying generalized seizure is a challenging problem. Indeed, seizures in partial epilepsy could be localized in the brain, and may be associated with anatomical changes. In contrast, generalized seizures appear to start from diffuse and bilateral areas in the brain, and are not associated with detectable changes in the brain structure (Dichter 1997). Based on both animal models and human studies, four main theories have been proposed:

1) *the centrencephalic theory*: Jasper and Kershman first proposed that generalized spike-and-wave discharges (GSW), the typical electroencephalographic (EEG) feature of IGE, originate from the subcortical structures (Jasper and Kershmann 1941). This hypothesis was based on two observations: abrupt onset and termination of GSW found simultaneously in both hemispheres, and high interhemispheric synchronization of the GSW discharges. These investigators later demonstrated that stimulation of intralaminar nuclei of the thalamus in cats produce bilaterally synchronous SWD on EEG recordings;

2) *the cortical theory*: the centrencephalic theory was later challenged, when Benett and Gloor showed that injection of a pro-convulsive drug (pentylenetetrazol) in the carotid artery (supplying the cerebral cortex) may produce GSW in patients with absence

seizures, whereas injection of the same drug in the verterbral artery (supplying the thalamus and brainstem) failed to do so (Bennett 1953; Gloor 1968);

3) *the cortico-reticular theory*: by using the feline penicilline generalized epilepsy model, Gloor reproduce SWD by diffuse application of penicilline onto the cortex, whereas injection of the same drug in the thalamus failed to do so (Gloor 1968). However, the integrity of the thalamocortical projections in this model appears to be essential in order to generate the SWD. Phase-locked firing started a few cycles earlier in the cortex than in the thalamus. Overall these data give rise to the *corticoreticular theory*, suggesting that the cortex first initiates the paroxysmal oscillations of the SWD burst and secondarily entrains the thalamus (Meeren et al. 2005);

4) *the cortical focus theory*: Meeren *et al* used field potentials to record simultaneously multiple cortical and thalamic sites in a rat strain (WAG/Rij) with genetic predisposition to absence seizures and GSW. By using this technique, they found that GSW discharges originate form the perioral region of the somatosensory cortex, spread to other cortical areas, and eventually to the thalamic nuclei (Meeren et al. 2002). According to these results, a 'cortical' focus is playing a leading role in the generation of GSW. This initial discharge propagates through cortico-cortical netrworks, before initiating the paroxysmal oscillation within the corticothalamic loops (Meeren et al. 2005).

More recently, Gotman and collaborators used a combination of EEG and functional MRI (fMRI) [blood oxygenation level-dependent (BOLD) effect] in order to study the haemodynamic response during GSW in humans. These authors found that, during GSW

activity fMRI changes, either in the form of activation (increased BOLD) or deactivation (decreased BOLD), occurred symmetrically in the cortex of both hemispheres (Aghakhani et al. 2004). Bilateral thalamic changes were also found in the majority of the patients from this study thereby confirming, in a group of human patients, the evidence of thalamic involvement seen in animal models for the generation of GSW.

Molecular basis for generalized spike-wave discharges

Whether the original SWD originate from the cortex or the thalamus, it is clear from these studies that the integrity of both cortical and thalamic structures is needed in order to propagate and maintain SWD. Thalamic neurons have the unique ability to shift between the oscillatory and tonic firing mode. The alert behavioural state is characterized by a desynchronized EEG pattern, which is associated with tonic firing of thalamocortical neurons. Conversely, when the firing patterns of these neurons shift to an oscillatory, rhythmic, burst firing mode, signal transmission to the cortex is dampened, and consciousness is depressed (Steriade et al. 1990; Steriade et al. 1993; Snead 1995). This oscillatory neuronal behaviour is an intrinsic ability of neurons whithin the nucleus reticularis thalami (NRT), which is composed of GABAergic neurons that project heavily to thalamic relay nuclei (Snead 1995). The cellular events underlying the ability of NRT neurons to shift between an oscillary and firing mode is the calcium low-threshold spike, which is triggered by $GABA_B$-mediated late inhibitory postsynaptic potentials (IPSPs) (von Krosigk et al. 1993). This finding is consistent with the observation that the most effective drugs against absence seizures (ethosuximide, benzodiazepine, and valproic acid) exert their effect directly or indirectly on the T-type calcium current within the thalamus (Snead 1995). Finally, cholinergic, dopaminergic, and noradrenergic

projections to cerebral cortex and thalamus may also modulate the GSW discharges (Snead 1995).

GENETIC MODELS OF EPILEPSY

In addition to epilepsy induced by administration of pro-convulsive drugs or by electrical stimulation of the brain, spontaneous epilepsy can also be observed in a variety of animal models. More specifically, an increasing number of genes have been identified in spontaneous mouse mutants (Table 4). Mutations in these genes have been associated with a variety of phenotypes, and may be associated with additional neurological manifestations, such as abnormal gait, movement, and posture (Noebels et al. 1997; Meisler et al. 2001; Noebels 2003). In particular, missense and nonsense mutations in four of the 24 voltage-gated Ca2+ channel subunit genes (α1a, β4, α2δ, γ2) have been associated with epilepsy and spike-wave discharges in mouse. Interestingly, mutations in two of these genes (*CACNB4*, *CACNA1A*) have also been associated with idiopathic generalized epilepsy in Humans (Escayg et al. 2000a; Chioza et al. 2001; Jouvenceau et al. 2001). Whereas other epilepsy genes found in mice could be responsible for human epilepsies remain to be determined. However, truncation of *MASS1*, a gene encoding for a membrane protein, was found in the Frings mouse model, which is characterized by seizures induced by loud noises (audiogenic) (Skradski et al. 2001). This gene may be functionally related to *LGI1* gene, which is mutated in autosomal dominant partial epilepsy with auditory features (ADPEAF) in humans (see p. 41). Finally, *stargazer* mice exhibit absence epilepsy, associated with spike-and-waves discharges and ataxia, which is caused by mutation in stargazin, a transmembrane protein that regulates AMPA receptors trafficking and gating (Nicoll et al. 2006).

Table 4. Spontaneous mutations associated with epilepsy in mice

Symbol Gene	Chr	Protein	Other Phenotypes
1) Ion channels and membrane excitability			
Cacna1a*	8	calcium channel, α1A subunit	
Cacna2d2	9	calcium channel, α2/δ2 subunit	growth/size, reproductive, lethality/postnatal
Cacnb4*	2	calcium channel, β4 subunit	muscle
Cacng2	15	calcium channel, γ2 subunit	growth/size, reproductive
Kcna1*	6	potassium channel, shaker-related subfamily, member 1	growth/size, vision/eye
Slc9a1	4	solute carrier family 9 (sodium/hydrogen exchanger)	lethality/postnatal, life span/aging, growth/size, behavior, nervous system
Szt1	2	genomic deletion (Kcnq2, Chrna4, Arfgap1)	growth/size
2) Other mechanisms			
Gpr98	13	G protein-coupled receptor 98 (Mass1)	hearing/vestibular/ear,
Atrn	2	attractin	pigmentation, nervous system, growth/size, adipose, behavior, skin/coat/nails, muscle
Pldn	2	pallidin	life span/aging, pigmentation, respiratory, homeostasis, renal/urinary, hearing/vestibular/ear, hematopoietic, immune

*gene associated with epilepsy in Humans

Source: http://www.informatics.jax.org/

In addition to spontaneous genetic models, epilepsy phenotype has been documented in mice following targeted mutagenesis, either incidentally or because of the implication of specific gene in human epilepsy. So far, more than 111 genes disrupted in mice (knock-out) have been associated with epilepsy phenotype. Disrupted genes associated with a phenotype close to idiopathic epilepsy are listed in Table 5. Overall, these genes can be divided into three functional categories, including: 1) ion channel and membrane excitability; 2) synaptic signalling; and 3) other mechanisms (e.g. neuronal plasticity, metabolism, network development). Again, some of these disrupted genes associated

with epilepsy phenotype in mouse may also cause epilepsy in Humans. Therefore, the identification of epilepsy genes, either in spontaneous or in transgenic mouse models, may accelerate the identification of genes causing epilepsies in Humans (Noebels 2003).

Table 5. Disrupted genes (knock-out) associated with epilepsy in mice

Symbol Gene	Chr	Protein	Other phenotypes
1) Ion channels and membrane excitability			
Cacna2d2	9	calcium channel, α2/δ2 subunit	life span/aging, cardiovascular, growth/size, homeostasis, immune, muscle, respiratory, hematopoietic
Kcna1*	6	potassium channel, shaker subfamily, member 1	life span/aging
Kcna4	2	potassium channel, shaker subfamily, member 4	
Kcnab2	4	potassium channel, shaker subfamily, β member 2	life span/aging, homeostasis
Kcnc1	7	Potassium channel, Shaw subfamily, member 1	muscle
Kcnc2	10	potassium channel, Shaw subfamily, member 2	muscle
Kcnc3	7	potassium channel, Shaw subfamily, member 3	muscle
Kcnj6	16	potassium channel, subfamily J, member 6	life span/aging
Kcnj9	1	potassium channel, subfamily J, member 9	life span/aging
Kcnk2	1	potassium channel, subfamily K, member 2	life span/aging
Kcnmb4	10	potassium channel, subfamily M, β member 4	
Kcnq2*	2	potassium channel, subfamily Q, member 2	
Scn1a*	2	sodium channel type I, α subunit	lethality/postnatal
Scn1b*	7	sodium channel type I, β subunit	lethality/postnatal, growth/size, vision/eye
Scn2b	9	sodium channel type II, β subunit	
Slc12a5	2	solute carrier family 12, member 5	lethality/postnatal, growth/size
Slc13a1	6	solute carrier family 13 (sodium/sulphate symporters), member 1	growth/size, homeostasis, renal/urinary, reproductive
Slc1a2	2	solute carrier family 1 (glutamate transporter), member 2	life span/aging, growth/size
Slc1a3	15	solute carrier family 1 (glutamate transporter), member 3	hearing/vestibular/ear
Slc2a1	4	solute carrier family 2 (glucose transporter), member 1	
Slc4a3	1	solute carrier family 4 (anion exchanger), member 3	
Gja12	11	gap junction membrane channel protein, α12	life span/aging, vision/eye
Gjb1	X	gap junction membrane channel protein, β1	life span/aging, vision/eye

*gene associated with epilepsy in Humans

Table 5. Disrupted genes (knock-out) associated with epilepsy in mice (continued)

Symbol Gene	Chr	Protein	Other phenotypes
2) Synaptic signalling			
Adam22	5	disintegrin and metallopeptidase domain 22	lethality/postnatal, growth/size
Chrna4*	2	cholinergic receptor, nicotinic, α4 subunit	
Chrna5	9	cholinergic receptor, nicotinic, α5 subunit	
Chrna7	7	cholinergic receptor, nicotinic, α7 subunit	immune
Chrnb4	9	cholinergic receptor, nicotinic, β4 subunit	
Gabbr1	17	gamma-aminobutyric acid (GABA-B) receptor, 1	homeostasis, touch/vibrissae
Gabbr2	4	gamma-aminobutyric acid (GABA) B receptor 2	homeostasis, touch/vibrissae
Gabrb3	7	gamma-aminobutyric acid (GABA-A) receptor, β3 subunit	lethality/embryonic-perinatal, craniofacial, growth/size, digestive
Gria2	3	glutamate receptor, ionotropic, AMPA2 (α2)	lethality/postnatal, growth/size
Gria4	9	glutamate receptor, ionotropic, AMPA4 (α4)	touch/vibrissae
Grik2	10	glutamate receptor, ionotropic, kainate 2 (β2)	growth/size
Grin2a	16	glutamate receptor, ionotropic, NMDA2A (ε1)	
Grm7	6	glutamate receptor, metabotropic 7	
Htr1a	13	5-hydroxytryptamine receptor 1A	
Htr2c	X	5-hydroxytryptamine receptor 2C	life span/aging, growth/size, adipose
Htr4	18	5 hydroxytryptamine receptor 4	lethality/embryonic-perinatal
3) Synaptic vesicles release apparatus			
Ap3b2	7	adaptor-related protein complex 3, β2 subunit	
Ap3m2	8	adaptor-related protein complex 3, μ2 subunit	
Sv2a	3	synaptic vesicle glycoprotein 2a	lethality/postnatal, growth/size
Sv2b	7	synaptic vesicle glycoprotein 2b	lethality/postnatal
Syn1*	X	synapsin I	
Syn2	6	synapsin II	
Synj1	16	synaptojanin 1	lethality/postnatal, growth/size

*gene associated with epilepsy in Humans

Table 5. Disrupted genes (knock-out) associated with epilepsy in mice (continued)

Symbol Gene	Chr	Protein	Other phenotypes
4) Other mechanisms			
Adarb1	10	adenosine deaminase, RNA-specific	lethality/postnatal
Amph	13	amphiphysin	life span/aging
Ank3	10	ankyrin 3, epithelial	life span/aging
Ckb	12	creatine kinase, brain	
Cnr1	4	cannabinoid receptor 1 (brain)	life span/aging
Cntn2	1	contactin 2	
Cplx1	5	complexin 1	life span/aging, reproductive
Dgke	11	diacylglycerol kinase, epsilon	
Fosb	7	FBJ osteosarcoma oncogene B	
Fyn	10	Fyn proto-oncogene	life span/aging
Fzd9	5	frizzled homolog 9 (Drosophila)	
Gad2	2	glutamic acid decarboxylase 2	life span/aging
Gad2	2	glutamic acid decarboxylase 2	life span/aging, digestive, endocrine, growth/size, immune
Gpr98	13	G protein-coupled receptor 98	life span/aging
Mt3	8	metallothionein 3	
Nidl	13	nidogen 1	
Plcl1	1	phospholipase C-like 1	life span/aging
Unc13b	4	unc-13 homolog B (C. elegans)	
Usf2	7	upstream transcription factor 2	growth/size

Source: http://www.informatics.jax.org/

EVIDENCE FOR A GENETIC ETIOLOGY TO EPILEPSY IN HUMANS

At least three lines of evidence suggest a genetic component to idiopathic epilepsies in Humans, including: 1) consistent observation of an increased risk for epilepsy in relatives and offspring of proband with various epileptic syndromes; 2) higher concordance rate for epilepsy in monozygotic twins compared to dizygotic twins, and 3) successful mapping and cloning of genes causing rare Mendelian forms of idiopathic partial and generalized epilepsies, also called *monogenic epilepsies*. This latter issue will be discussed in a separate section (see p. 37).

Familial aggregation studies

Lennox (1951) first observed that generalized spike and waves discharges (GSW), the classical EEG pattern associated with IGE, aggregates in families (Lennox 1951; Pedley 1997). Following this original finding, familial aggregation studies consistently showed a threefold increased risk of epilepsy (~3%) in siblings of probands with idiopathic or cryptogenic epilepsy, compared to the general population (Annegers et al. 1982; Hauser 1997; Jain et al. 1997). In contrast, symptomatic epilepsies do not increase this risk. A higher risk (6 to 8%) is observed for individuals with idiopathic generalized epilepsy associated with typical GSW. In addition, the occurrence of GSW in another member of the family (parent or sib) further increases the risk in the sibling, respectively to 12 and 15%. Finally, analyses of epileptic individuals' offspring have shown a higher risk in offspring from parents with absences seizures (9%) than those with other generalized (2.3%) or partial seizures (2.7%) (Hauser 1997).

The risk of febrile seizure (FS) is also increased in siblings of proband with FS (6-10%) compared to the general population (2-3%) (Hauser et al. 1985). The sibling's FS risk rises to 22% when one parent of the proband is affected and to 56% when both parents have FS. Finally, the occurrence of febrile seizure associated with epilepsy in the proband significantly increase the risk for epilepsy in siblings (6%) (Hauser 1997).

Twin studies

Several studies of epilepsy in twins have consistently shown a higher concordance rate for the disease in monozygotic (MZ) pairs compared with dizygotic (DZ) pairs, providing additional support for a significant genetic component to the common epilepsies (Inouye

1960; Marshall et al. 1962; Schiottz-Christensen 1972; Corey et al. 1991; Sillanpaa et al. 1991). However, the concordance rate was highly variable from one study to the other. A higher concordance rate in identical twins has been observed from referred or hospital series compared to community-based studies (Berkovic et al. 1998). The difference in the study designs may have introduced an ascertainment bias, since twin pairs with both affected individuals are more likely to be referred to the investigator. In addition, the difference in the genetic contribution between the various epileptic syndromes could not be appreciated from these earlier twin studies, because they have been performed before the contemporary classification of the epileptic seizures and syndromes.

In a study of epilepsy in a twin cohort from Australia, Berkovic *et al* addressed both the issue of population based versus referred twin series, by using contemporary classifications of epilepsy syndromes (Berkovic et al. 1998). These authors observed a high rate of false positive diagnosis for epilepsy in the register sample, suggesting that community-based studies based on self-reports may be unreliable. In addition, referred twins have an excess of severe cases and exhibit higher concordance rate (MZ = 0.61; DZ = 0.15) compared to registered twins (MZ = 0.34; DZ = 0.07), confirming the existence of ascertainment bias when using a referred twin population. Consistent with the observation made from familial aggregations studies, the casewise concordances for idiopathic generalized epilepsies (MZ = 0.76; DZ = 0.33) were greater than those for partial epilepsies (MZ = 0.36; DZ = 0.05), with intermediate values for febrile seizures (MZ = 0.58; DZ = 0.14). These data further support that idiopathic generalized epilepsies has an important inherited component. In turn, the heritable component in the partial epilepsies is smaller than in IGE, suggesting that acquired factors are playing a greater

role in these epileptic syndromes. Interestingly, in 94% of concordant MZ pairs, affected twins exhibit the same epilepsy syndrome, suggesting the existence of syndrome-specific genetic determinants rather than a broad genetic predisposition to seizures (Berkovic et al. 1998). More recently, a reappraisal of Lennox's series of twin pairs using modern classification of epileptic syndromes led to the same conclusions than the Australian series (Vadlamudi et al. 2004).

Complex inheritance patterns in idiopathic epilepsies

Both familial aggregation and twin studies are strongly suggestive of a genetic component in epilepsy. However, we have to keep in mind that twin pairs and family relatives not only share genes, but also many predisposing factors from the environment, as well as behavioural practices (Ottman 1997). To my knowledge, neither familial aggregation nor twin studies studies in epilepsy have so far controlled for these shared environmental factors. In turn, numerous environmental factors have been implicated as risk factors or precipitants for seizure disorders including stress and mood disorders, sleep deprivation, alcohol and drug consumption, head trauma, and stroke (Hauser 1997; Loiseau 1997). It is thus possible that some environmental factors contribute to the observed familial aggregation and high concordance rates observed in twins.

In rare families, autosomal dominant inheritance of epilepsy and/or febrile seizures occurs (see p. 37). However, clinical observations indicate that, in the vast majority of the families, a single-gene mode of inheritance is implausible, and complex inheritance involving two or more genes is most likely (Rich et al. 1987; ILAE 1993; Marini et al. 2004). These clinical observations are further supported by genetic epidemiology studies

(Annegers et al. 1982; Hauser 1997; Jain et al. 1997; Berkovic et al. 1998). Indeed, the relative risk of being affected for a relative of an individual with IGE (λ_R) is almost 100 for monozygotic twins (λ_M), 7.5 for sibs (λ_S), and 1.5 for nieces/nephews (λ_N). For idiopathic and cryptogenic partial epilepsy, the relative risk of being affected for a relative (λ_R) is 55 for monozygotic twins (λ_M), 3.5 for sibs (λ_S), and 1.5 for nieces/nephews (λ_N). Such a rapid decrease in the λ_R with each degree of relationship suggests a multiplicative (epistatic) interaction among many contributing loci for idiopathic generalized and partial epilepsy (Risch 1990; Zara et al. 1995). However, these epidemiological studies probably represent a crude approximation of a very complex reality. Indeed, these studies lumped all the generalized or partial epilepsy syndromes together, even though they have been shown to be clinically, and genetically highly heterogeneous. In addition, the λ_R is probably underestimated in the majority of these studies because of the difficulties inherent to the assessment of epilepsy phenotype, in both the proband and their relatives.

Difficulties inherent to the study of genetic epilepsies in humans

So far, genes underlying monogenic epilepsies have been mapped (and eventually cloned) by using conventional linkage methods. This is a robust, powerful and well established method that allowed the identification of many genes causing different diseases in Humans (Eaves et al. 2000). In order to achieve sufficient power of analysis, the critical step in this experimental approach is the identification of large kindreds with many affected individuals, for which a clear mode of inheritance can be established (e.g. autosomal dominant, recessive, X-linked). However, the identification and clinical

characterization of such families is not an easy task. Indeed, several difficulties inherent to the study of epilepsy have impeded progress in the identification genes predisposing to the disease (Ottman 1997), including:

(1) epilepsy is a highly heterogeneous disease, and a wide range of clinical manifestations within the same family is often observed (Scheffer and Berkovic 1997; Xiong et al. 1999; Picard et al. 2000). Therefore, the *a priori* definition of a phenotype caused by a specific group of genes remains a challenge;

(2) epilepsy is not an 'all-or-none' phenomenon, and the investigators eventually have to determine who will be considered as affected among the family members exhibiting intermediate phenotypes, such as single seizure, febrile seizure, or isolated electroencephalographic (EEG) abnormalities (Scheffer and Berkovic 1997; Greenberg et al. 2000);

(3) both clinical and EEG manifestations may vary according to the age of the individual (e.g. age-dependant penetrance of generalized spike-and-waves) (Metrakos and Metrakos 1961), thereby limiting the time window in which the investigator can directly assess the clinical syndrome;

(4) because of this wide range of clinical manifestations, definitions for the disease are often ambiguous and varied from one investigator to the other;

(5) the cornerstone in the diagnosis of epilepsy is a careful medical history. In practice, this evaluation is not always possible in all affected members of the family. Information on these relatives is often obtained indirectly, by interviewing the proband or its parents, which can be misleading;

(6) especially in older generations of the family, social issues may contribute to an underestimation of affected individuals within a family, including various stigmas and misconceptions about the origin of the disease, and guilty of "passing on" a disorder;

(7) even with a systematic survey of all affected individuals within a family, the penetrance of many familial epilepsies remain consistently low (Scheffer and Berkovic 1997; Xiong et al. 1999; Picard et al. 2000);

(8) both underreported cases and a reduced penetrance within families make difficult the identification of large pedigrees with many affected individuals;

(9) overall, the prevalence of epilepsy is relatively high in the general population (1%), and could be even higher in intermediate phenotypes, such as febrile seizures (5%), and photoconvulsive response (8%) (Eeg-Olofsson et al. 1971; Doose and Gerken 1973). Such a high phenocopy rate may introduce a significant risk of a false negative linkage;

Because of all these potential difficulties, the identification of genes underlying monogenic epilepsies in Humans is challenging. While many of these caveats are inherent to problems in the clinical assessment of the disease, many other difficulties are probably related to the complexity of the underlying biological mechanisms.

MONOGENIC EPILEPSIES IN HUMANS

Despite these above mentioned difficulties, the identification of large families segretating epilepsy as a Mendelian trait allowed the successful mapping and eventually led to the identification of mutations in several genes for the disease. One of the critical steps in the identification of epilepsy genes was the recognition of specific inherited epileptic syndromes. These successful examples are presented in Table 7. Additional putative loci for epilepsy genes can be found in Table 6.

Monogenic generalized epilepsies

Benign neonatal familial convulsions

Rett and Teubel reported in 1964 the first family with benign neonatal familial convulsion syndrome (BNFC), an autosomal dominant syndrome subsequently recognized by several independent authors (Rett and Teubel 1964; Plouin and Anderson 2002) and acknowledged in the International Classification of the Epilepsies (ILEA). Clinical manifestations of BNFC were described in p. 7. Historically, BNFC was the first epilepsy for which a gene could be localized, and mutations in *KCNQ2* and *KCNQ3*, encoding for two different subunits of the same potassium channel, have been asscociated with the disease (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998).

Idiopathic generalized epilepsy with febrile seizures

More recently, Scheffer *et al* identified a *hitherto* unrecognized idiopathic generalized epilepsy syndrome following single gene inheritance, named *generalized epilepsy with febrile seizures plus* (GEFS+) (Scheffer and Berkovic 1997). In these families, the most common clinical manifestation was febrile seizure. However, unlike typical febrile seizures (FS), attacks with fever may continue beyond six years of age and were called febrile seizure plus (FS+). In most affected individuals, FS or FS+ were eventually followed by afebrile seizures, including absences, myoclonic, and atonic seizures. The most severely affected individuals have myoclonic-astatic epilepsy associated with mental retardation. In these GEFS+ families, the pattern of inheritance was autosomal dominant, and the large variation in generalized epilepsy phenotypes was not explained by acquired factors. Recognition of this syndrome allowed the identification of at least three genes for GEFS+: the $\beta 1$ and $\alpha 1$ subunits of the neuronal voltage gated sodium

channel (*SCN1B, SCN1A*) (Wallace et al. 1998; Escayg et al. 2000b) and the γ2 subunit of $GABA_A$ receptor (*GABRG2*) (Baulac et al. 2001; Wallace et al. 2001a). However, the mechanism by which these mutations in *SCN1A* lead to spontaneous and uncontrolled discharges of neuronal cells remains to be determined. *In chapter 2 of this thesis, the identification of a new D188V mutation in SCN1A in the original family with GEFS described by Scheffer et al, which has been previously mapped to chromosome 2q23-24* (Lopes-Cendes et al. 2000), *will be presented. The functional consequence of this mutation on sodium channel currents recorded in vitro will also be described.*

Autosomal dominant idiopathic generalized epilepsy

Rare families segregating classical idiopathic generalized epilepsy syndromes have been recently described. Mutations in the voltage-gated chloride channel type 2 (*CLCN2*) were associated with CAE, JAE, JME, and EGMA (Haug et al. 2003), whereas mutations in EFHC were found in families with classical JME (Suzuki et al. 2004). In addition, mutations in the δ subunit of the $GABA_A$ receptor (*GABRD*) were found in small nuclear families with GEFS, CAE and JME (Dibbens et al. 2004). *In chapter 3 of this thesis, a large family segregating juvenile myoclonic epilepsy with autosomal dominant inheritance caused by a mutation in GABRA1 will be described.*

So far, a total of six additional loci have been mapped for generalized epilepsy syndromes in which Mendelian inheritance is suspected (Zara et al. 1995; Elmslie et al. 1997; Fong et al. 1998; Sander et al. 2000; Baykan et al. 2004; Puranam et al. 2005), three putative loci for photosensitivity trait (Pinto et al. 2005; Tauer et al. 2005), as well as four loci for febrile seizures (Wallace et al. 1996; Johnson et al. 1998; Nabbout et al.

2002; Deprez et al. 2006) (Table 6). The identification of these susceptibility genes is pending.

Table 6. Loci associated with idiopathic generalized epilepsy in Humans

Phenotype	Inheritance	Locus	Reference
1) Infantile generalized epilepsy			
BIFC	AD	19q	Guipponi, 1997
BMEI	AR	16p13	Zara, 2000
2) Classical generalized epilepsy			
CAE	AD	8q24	Fong, 1998
CAE/EIG1	-	8q24	Zara, 1995
JME	-	15q14	Emslie, 1997
IGE/EIG2	AD	14q23	Sander, 2000
IGE/EIG3	AR	9q32-32	Baykan, 2004
IGE/EIG4	AD	10q25-26	Puranam, 2005
3) Photosensitivity trait			
PPR1	AR	6p21	Tauer, 2005
PPR2	AD	13q31	Tauer, 2005
PPR3	AD	7q32	Pinto, 2005
4) Adult-onset generalized epilepsy			
FAME 1	AD	8q24	Mikami, 1999
FAME 2	AD	2p11.1-q12.2	Guerrini, 2001
5) Partial epilepsy			
ADNFLE	AD	15q13	Phillips, 1998
FTLE/ETL2	AD	12q22-23	Claes, 2004
FPEVF	AD	22q11-12	Xiong, 1998
PEPS	AD	4p15	Kinton, 2002
6) Febrile seizures			
FEB1	AD	8q13-21	Wallace, 1996
FEB2	AD	19p	Johnson, 1998
FEB3	AD	6q22-24	Nabbout, 2002
FEB4	AD	5q14-23	Deprez, 2006
6) Others			
REPED/PKC/FICPC	AR	16p12-11	Guerrini, 1999

Monogenic partial epilepsies

Autosomal dominant nocturnal frontal lobe epilepsy

Idiopathic partial epilepsies with Mendelian inheritance have also been described. The first example is autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), which has been described by Scheffer *et al*, based on a group of five families with a homogeneous phenotype (Scheffer et al. 1995). The epilepsy usually began in childhood, and persisted through adult life, with considerable intra-family variation in severity. Affected individuals were characterized by clusters of brief nocturnal motor seizures, with hyperkinetic or tonic manifestations, typically as the individual dozed, or shortly before awakening. Seizures were often misdiagnosed as benign nocturnal parasomnias, psychiatric and medical disorders, and the syndrome was initially described as nocturnal paroxysmal dyskinesia. Interictal EEG and neuro-imaging studies were consistently normal. In turn, ictal video-EEG studies showed that the attacks were partial seizures originating from the frontal lobe. This disorder showed autosomal dominant inheritance with reduced penetrance. Many of these ADNFLE families are caused by mutations in *CHRNA4*, *CHRNA2*, and *CHRNB2*, encoding for three different subunits of the ligand-gated neuronal nicotinic acetylcholine receptor ($\alpha4$, $\alpha2$, $\beta2$) (Steinlein et al. 1995; De Fusco et al. 2000; Aridon et al. 2006). Other ADNFLE families have been mapped to chromosome 15, in a region encoding for a cluster of nicotinic acetylcholine receptor, but the identification of causative mutations in these families is pending (Phillips et al. 1998).

Autosomal dominant partial epilepsy with auditory features

Autosomal dominant partial epilepsy with auditory features (ADPEAF) was mapped to chromosome 10q24 (Ottman et al. 1995; Winawer et al. 2000). In affected individuals,

auditory hallucinations were the most striking clinical manifestation, but other sensory symptoms (e.g. visual, olfactory, vertiginous, and cephalic) were reported. Autonomic, psychic, and motor symptoms were less common. Although interictal EEG were invariably normal, the clinical semiology points to a lateral temporal seizure origin. In the majority of affected individuals, neuro-imaging is normal. In rare cases, cortical dysplasias involving the temporal neocortex have been reported (Kobayashi et al. 2003). Nonsense mutations in leucine-rich gene, glioma-inactivated-1 (*LGI1*) have been found in families segregating ADPEAF (Kalachikov et al. 2002).

Familial temporal lobe epilepsy (FTLE) (Berkovic et al. 1996; Depondt et al. 2002; Claes et al. 2004), familial epilepsy with variable foci (FPEVF) (Xiong et al. 1999), and partial epilepsy with pericentral spikes (PEPS) (Kinton et al. 2002) are additional partial epilepsy syndromes in which Mendelian inheritance is suspected (Table 6). However, the identification of genes underlying these putative monogenic syndromes is pending. Finally, Garcia *et al* described a nonsense mutation in *SYN1*, encoding for synapsin 1, in a family segregating a mixture of neurodevelopmental disorders, including learning disorders, aggressive behaviour and epilepsy in some cases (Garcia et al. 2004). *In chapter 4 of this thesis, additional evidences for the existence of this latter form of monogenic partial epilepsy will be provided, with the description of a large kindred showing recessive X-linked temporal lobe epilepsy caused by mutation in SYN1.*

Table 7. Genes associated with idiopathic epilepsy in Humans

Phenotype	Inheritance	Locus	Gene	Reference
1) Potassium channels				
BNFC	AD	20q13	KCNQ2	Biervert, 1998
BNFC	AD	8q24	KCNQ3	Charlier, 1998
EA1/epilepsy	AD	12p13	KCNA1	Eunson, 2000
GEPD	AD	10q22	KCNMA1	Du, 2005
2) Sodium channels				
GEFS	AD	2q24	SCN1A	Escayg, 2000
GEFS	AD	19q13	SCN1B	Wallace, 1998
BFNIC	AD	2q22	SCN2A	Heron, 2002
3) Chloride channels				
JME/JAE/EGMA	AD	3q26	CLCN2	Haug, 2003
4) Calcium channels				
JME	-	2q22-23	CACNB4	Escayg, 2000
IGE/EA2/FHM	-	19p13	CACNA1A	Chioza, 2001
CAE	-	16p13	CACNH1	Chen, 2003
5) $GABA_A$ receptors				
JME	AD	5q34	GABRA1	Cossette, 2002
GEFS/CAE	AD	5q33	GABRG2	Wallace, 2001a
JME		1p36	GABRD	Dibbens, 2004
6) Nicotinic acetylcholine receptors				
ADNFLE	AD	1q21	CHRNB2	DeFusco, 2000
ADNFLE	AD	20q13	CHRNA4	Steinlein, 1995
ADNFLE	AD	8p21	CHRNA2	Aridon, 2006
7) Non-ion channel genes				
JME	AD	6p12-11	EFHC1	Suzuki, 2004
ADPEAF	AD	10q24	LGI1	Kalachikov, 2002
EVLD	XR	Xp11	SYN1	Garcia, 2004
JME	-	6p21	BRD2	Pal, 2003
IGE	-	18q21	MEA2	Greenberg, 2005

Paroxysmal neurological disorders associated with idiopathic epilepsy

Idiopathic epilepsies with Mendelian inheritance have been associated with other paroxysmal neurological disorders, including episodic ataxia type 1 and paroxysmal dyskinesia, respectively caused by mutations in *KCNA1* and *KCNMA1* (Eunson et al. 2000; Du et al. 2005). In addition, a putative locus has been identified for familial infantile convulsions with choreoathetosis (FICPC) (Szepetowski et al. 1997), a syndrome that is also named Rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp (REPED) (Guerrini et al. 1999) or epilepsy associated with paroxysmal kinesigenic choreoathetosis (PKC) (Sadamatsu et al. 1999).

Table 8. Monogenic epilepsy syndromes

- Benign neonatal familial convulsions
- Benign infantile familial convulsions
- Generalized epilepsy with febrile seizures plus
- Autosomal dominant idiopathic generalized epilepsy
- Autosomal dominant nocturnal frontal lobe epilepsy
- Autosomal dominant partial epilepsy with auditory features
- Familial temporal lobe epilepsy
- Familial partial epilepsy with variable foci

HYPOTHESIS AND OBJECTIVES

Genetic epidemiology studies thus indicate that, overall, the common forms of epilepsy in Humans are complex genetic traits. However, an increasing number of large kindred, segregating either partial of generalized epilepsy syndromes, have been recently identified,

and allowed the identification of specific genes causing Mendelian forms of epilepsy. The current state of knowledge thus indicates that idiopathic generalized (or partial) epilepsies are clearly not a single entity, but rather represent the sum of a large variety of different genetic diseases: some being clearly Mendelian traits, while others might exhibit polygenic inheritance. Therefore, to address such a complex neurological disorder, a combination of methodological approaches is needed, and should include the recruitment of a sufficient large number of families with detailed clinical evaluations. In particular, systematic survey of family members of proband with standardized questionnaires is needed in order to identify large kindred segregating epilepsy. By using this strategy, *our main hypothesis is that additional forms of familial epilepsy could be uncovered.* More specifically, the objective of this work is to identify additional genes that predispose to idiopathic epilepsies and begin to determine how mutations in these genes lead to the disease. In order to achieve these objectives, my specific aims are:

(1) identification of the gene causing an autosomal dominant form of IGE associated with febrile seizures plus (GEFS+) in a large Australian kindred;

(2) mapping and identification of the gene causing an autosomal dominant form of juvenile myoclonic epilepsy (JME) that we have identified in a large French-Canadian kindred.

(3) mapping and identification of the gene causing a familial form of temporal lobe epilepsy with recessive X-linked inheritance that we have identified in a large French-Canadian kindred;

(4) characterization of biochemical and electrophysiological properties of the gene mutations discovered in specific aims (1), (2) and (3).

PROPOSED EXPERIMENTAL PLAN

Different strategies can be used to map and identify genes predisposing to complex phenotypes in Humans (eg. non-parametric linkage analysis, association studies). *In this thesis, we will focus on studying familial forms of idiopathic epilepsy, mainly from the French-Canadian population.* For this purpose, we propose to use the classical methods that have been shown to be efficient in Human Genetics, including:

(1) identification of familial cases with epilepsy, with an emphasis on large kindreds;

(2) detailed clinical evaluation of affected individual in order to identify distinct clinical syndromes;

(3) blood sampling, DNA extraction and establishment of lymphoblastoid cell lines;

(4) genotyping and linkage analysis;

(5) sequencing of candidate genes mapping to the linked region; and

(6) functional characterization of the mutant versus normal genes.

This approach has been found to be successful for the identification of genes causing various neurological diseases, including rare forms of epilepsy. One potential limitation however is that we may find genes predisposing only for the monogenic forms of the disease, which are believed to be rare. However, the incidence of familial epilepsy is currently unknown, and is probably overlooked in clinical practice. For the study of epilepsy with complex inheritance, a large cohort of well-characterized families would be needed in order to perform non-parametric linkage studies (e.g. allele-sharing methods, TDT). This method may facilitate the identification of the genes with a smaller impact on the phenotype. These genes are probably responsible for a higher proportion of

idiopathic epilepsy. However, although the families recruited for this study could also be included in this latter analysis, *this approach will not be considered in this thesis.*

Should we map a new locus for familial epilepsy, the genes mapping to the minimal candidate interval will be retrieved from public databases and will be screened for mutation. Prior to screening, candidate genes will be prioritized based on a number of criteria, summarized as follows:

(1) expression in brain regions relevant to partial or generalized epilepsy, identified in expression databases and from scientific literature. Examples of such regions are hippocampal formation, amygdala, temporal of frontal lobe neocortex, and thalamus;

(2) potential biological relevance to epilepsy, inferred from basic mechanisms underlying generalized or partial epilepsy, in Humans or animal models;

(3) similarity of function or sequence with epilepsy genes previously identified in Humans or mice.

Based on these criteria, potential candidate genes for idiopathic generalized and partial epilepsies are summarized in Table 9. *In this thesis, should we map a new locus by linkage study, a high priority will be given to the screening of these genes, if present in our candidate region.*

IMPACT OF IDENTIFICATION OF EPILEPSY GENES

The treatment of the epilepsies remains symptomatic, with up to 30% of patient who continue to have intractable seizures despite an adequate pharmacological treatment. This represents considerable human and societal costs. The exact mechanism by which

neurons spontaneously start to fire synchronously resulting in either focal of generalized seizure is elusive. In addition, the mechanisms of action of many anti-epileptic drugs, as well as the different response rate to these drugs among individuals are still poorly understood. We believe that the identification and the characterization of the mutated molecules involved in human epilepsies will enhance our understanding of the disease. In particular, identification of susceptibility genes for the epilepsies will allow the construction of transgenic animals that will facilitate the development of specifically targeted drugs. In addition, with a better understanding of the mode of inheritance and of the genetic etiology of the epileptic syndromes, we can expect to eventually offer better genetic counselling to the individuals with epilepsy and their family members, including DNA-based diagnostic tests. Eventually, early identification of individuals who are prone to develop temporal lobe epilepsy may provide a unique opportunity during which potentially neuroprotective treatments could be applied.

Table 9. Broad categories of candidate genes for epilepsy

(1) ion channel and ligand-gated ion channel receptors
(2) glutamatergic, cholinergic and gabaergic receptors
(3) enzymes involves in synthesis or metabolism of glutamate, GABA, or other neurotransmitter;
(4) proteins involved in neurotransmitter release machinery;
(5) proteins involves in neuronal development (e.g neurotrophins)

CHAPTER 2: FUNCTIONAL CHARACTERIZATION OF THE D188V MUTATION IN NEURONAL VOLTAGE-GATED SODIUM CHANNEL CAUSING GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS (GEFS)

Reference :

Cossette P, Loukas A, Lafrenière RG, Harvey E, Rochefort D, Ragsdale DS, Dunn R, and Rouleau GA. Functional characterization of the D188V mutation in *SCN1A* causing generalized epilepsy with febrile seizures plus (GEFS). *Epilepsy Research* 2003; 53: 107-17.

Preface

"...although I shall continue to speak for the most part of epilepsy as if there were such a clinical entity, there are really many different epilepsies..."
Hughlings Jackson, 1887

In this chapter, we present the identification and functional analysis of a D188V mutation in the α1 subunit of the voltage-gated sodium channel (*SCN1A*) in a large pedigree, the original family from which the syndrome of Generalized Epilepsy with Febrile Seizures plus (GEFS+) was described (Scheffer and Berkovic 1997). D[r] Rouleau's laboratory previously mapped the gene for this family to chromosome 2q23-31 in a region containing a cluster of genes encoding for sodium channel (Lopes-Cendes et al. 2000). While we were screening these genes for mutation, another group reported mutations in *SCN1A*, in two French families (Escayg et al. 2000b). This finding prompted us to re-examine our data. Meanwhile, mutations in *SCN1A* gene have been recognized as an important cause of familial epilepsy, with a variety of phenotypes. However, the functional consequences of these mutations remained largely unknown at that time. In this study, we found that sodium channels harbouring the D188V mutation were more resistant to the decline in amplitude that is normally observed over the course of high frequency pulse trains. This change in channel function is compatible with an increase in membrane excitability, such as during sustained and uncontrolled neuronal discharges. Our data suggest that this specific effect on sodium channel function could be a general mechanism in the pathophysiology of epilepsies caused by mutations in sodium channels in humans. From a clinical point of view, the variety phenotypes associated with mutations in *SCN1A* challenged the current classification of epilepsy syndromes.

Abstract

Mutations in the alpha 1 subunit of the voltage-gated sodium channel (*SCN1A*) have been increasingly recognized as an important cause of familial epilepsy in humans. However, the functional consequences of these mutations remain largely unknown. We identified a mutation (D188V) in *SCN1A* segregating with generalized epilepsy with febrile seizures (GEFS) in a large kindred. Compared to wild-type sodium channels, in vitro expression of channels harboring the D188V mutation were found to be more resistant to the decline in amplitude that is normally observed over the course of high frequency pulse trains. This small change on a single aspect of channel function is compatible with an increase in membrane excitability, such as during sustained and uncontrolled neuronal discharges. These data suggest that this specific effect on sodium channel function could be a general mechanism in the pathophysiology of epilepsies caused by mutations in sodium channels in humans.

Introduction

Epilepsy is a common neurological disorder, affecting approximately 1% of the population. Recently, genes underlying some of the idiopathic partial and generalized epileptic syndromes have been identified. The vast majority of the genes predisposing to familial epilepsy in humans incriminate ion channels. Hence, mutations have been found in voltage-gated sodium and potassium channels (*SCN1B*, *SCN1A*, *KCNQ2*, *KCNQ3*) (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998; Wallace et al. 1998; Escayg et al. 2000b), as well as in ligand-gated channels (*GABRA1*, *GABRG2*, *CHRNA4*, *CHRNB2*) (Steinlein et al. 1995; De Fusco et al. 2000; Baulac et al. 2001; Cossette et al.

2002). Among these epilepsy genes, mutations in the alpha 1 subunit of the voltage-gated sodium channel (*SCN1A*) are the most frequently encountered, and have been found to cause at least three different epileptic syndromes: generalized epilepsy associated with febrile seizure (GEFS) (Escayg et al. 2000b), febrile seizures associated with temporal lobe epilepsy (Sugawara et al. 2001a) and severe myoclonic epilepsy of infancy (Claes et al. 2001). However, even if *SCN1A* is increasingly recognized as an important gene causing epilepsy in humans, the mechanism by which these mutations in *SCN1A* lead to spontaneous and uncontrolled discharges of neuronal cells remains elusive. Several recent studies have assessed the functional effect of mutations in *SCN1A*, focusing on three mutations identified in families segregating GEFS (T875M, W1204R and R1648H) (Alekov et al. 2000; Alekov et al. 2001; Spampanato et al. 2001; Lossin et al. 2002). These functional studies suggest that sodium channel mutations contribute to neuronal hyperexcitability through subtle changes in persistent sodium current (Lossin et al. 2002), activation, inactivation or recovery from inactivation (Alekov et al. 2000; Alekov et al. 2001; Spampanato et al. 2001). In this study, we present the identification of a D188V mutation in *SCN1A* in a large family segregating GEFS that we have previously mapped to chromosome 2q23-24 (Lopes-Cendes et al. 2000). This mutation is located in the intracellular loop very close to the S3 transmembrane segment of domain I, which has no previously identified role in voltage-gating. Electrophysiological analysis of D188V mutant channels expressed in HEK cells shows that the mutation causes decreased cumulative inactivation of sodium current during high frequency channel activity, an effect compatible with membrane hyperexcitability. This mechanism could be central in the pathophysiology of the epilepsies caused by mutations in sodium channels in humans.

Materials and methods

Screening for mutation

Family collection and phenotypes have been described previously (Lopes-Cendes et al. 2000). The genomic organization of the human *SCN1A* gene was determined by partial sequencing of human PAC clones isolated from a chromosome 2 enriched PAC sublibrary (Gingrich et al. 1996) probed at low stringency with a rat *SCN2A* cDNA (Auld et al. 1988). Primers were designed to amplify 200–350 bp fragments from genomic DNA to screen all coding portions of the gene. Portions of the *SCN1A* gene were amplified by polymerase chain reaction (PCR) and analyzed by the single stranded conformational polymorphism technique (SSCP). We also performed direct sequencing of an affected patient and a control for all the coding region of the *SCN1A* gene. For exon 4, primers NaC-63, 5'-TTAGGGCTACGTTTCATTTGTATG-3'; and NaC-64, 5'-AGCACTGATGGAAAACCAAACTAT-3' were used for PCR and sequencing. PCR products showing a conformational change were reamplified from genomic DNA with the same primers, and sequenced using the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB). To study the segregation of the mutation found in the family, as well as for the screening of normal controls, we did PCR amplification as described earlier, followed by *Bam*HI digestion.

Mutagenesis

We used the QuickChange site-directed mutagenesis kit (Stratagene) to introduce the D188V mutation into cDNA encoding the rat voltage-gated sodium channel α subunit type 2 (*SCN2A)* that we have previously cloned in vector pEUK-CI (Clonetech,

Mississauga, Ont.) (Auld et al. 1988). Briefly, a truncated *SCN2A*-pEUK construct was created by digesting with *Sma*I and then circularizing a 6.9 kb fragment consisting of the entire vector and 5' and 3' segments of *SCN2A*. The mutation was introduced into this construct by PCR with pfu polymerase, using primers NaC-103 5'-TTGCCGTGTGCTATGACTTTGTGTTCTCAG-3', and NaC-104 5'-CTGAGAACACAAAGTCATAGCACACGGCAA-3', and religated with the remainder of the *SCN2A* cDNA at the *Sma*I site, to regenerate the full-length cDNA, containing the D188V mutation. Both the wild type and mutant constructs were subsequently sequenced, to confirm the presence of the mutation, and to rule out the introduction of spurious mutations that could have occurred during PCR.

Sodium channel expression and electrophysiological recording

To study sodium channel function, human embryonic kidney (HEK) cells were grown to 80% confluence in 60mm tissue culture dish in DMEM medium(GIBCO, Burlington, Ont.) and then transfected with 1.5 μg wild type or mutant *SCN2A*-pEUK DNA, along with 2 μg of *SCN1B* cDNA, encoding the α1subunit of the voltage-gated sodium channel, in vector pcDNA3.1-Zeo (Invitrogen, Burlington, Ont.) and 0.5 μg GFP in vector pEGFP-C1 (Clonetech), using Polyfect transfection reagent according to protocol supplied by the manufacturer (Qiagen, Mississauga,Ont.). Recordings were performed at room temperature 2–4 days after transfection, using the whole cell configuration of the patch clamp recording technique with an Axopatch 200 amplifier and pCLAMP software (Axon Instruments, Foster City, CA) (Hamill et al. 1981). Recording pipettes had resistances of 2–4 MΩ, and were filled with an intracellular solution consisting of (in

mM): 105 Cs-Aspartate, 10 CsCl, 10 NaCl, 10 EGTA and 10mM HEPES, pH 7.4 with CsOH. The extracellular bath solution contained (in mM): 130 NaCl, 4 KCl, 1.5 CaCl2, 1 MgCl2, 5 glucose and 10 HEPES, pH 7.4 with NaOH. Data were filtered at 5 kHz and sampled at 50 kHz. Capacitive currents were cancelled using the internal clamp circuitry. Remaining transient and leak currents were subtracted using the P/4 procedure. Series resistance compensation (using the clamp circuitry) was ≥70%. Series resistance errors were typically ≤2mV and were not greater than 5mV in any experiment used in our analysis. Experimental protocols and analysis for determining the voltage-dependence of channel activation, inactivation and recovery from inactivation as well as other details of whole cell recording were as described elsewhere (Kazen-Gillespie et al. 2000; Meadows et al. 2002). Statistically significant differences between wild type and mutant channels were determined by Student's *t*-test. Groups were considered significantly different when $P < 0.05$.

Results

Mutation screening

To identify mutation in our GEFS family, we initially performed SSCP analysis on three affected individuals from the pedigree, and on three normal controls using primers specific to the human *SCN1A* gene. A SSCP variant was detected in exon 4 with primers NaC-63 and NaC-64 for all of the affected patients, and none of the controls. No mutation was found in the other exons of the *SCN1A* gene. Sequence analysis determined that the patient was heterozygous for an A-T substitution predicted to change a GAT (Asp) to GTT (Val) codon at position 188 for both the rat (Noda et al. 1986) and human (Escayg

et al. 2000b) *SCN1A* cDNAs (Fig. 1A). This variation destroys a *Bam*HI restriction site, that we used as a test to screen all individuals from the family, as well as more control cases (Fig. 1B). The A-T substitution was seen in 17 affected and obligate carriers, but not in 10 unaffected individuals from this family. This mutation was not detected in more than 400 control chromosomes. Also the D188 residue is highly conserved in all sodium channels, in all species (Fig. 2).

Functional analysis of the D188V mutation

To determine the effects of the D188V mutation on sodium channel function, we introduced the mutation into cDNA encoding the rat ortholog of the voltage-gated sodium channel α subunit type 2 (*SCN2A*), and examined the functional properties of the expressed sodium channels by whole cell patch clamp recording. In all experiments, we coexpressed wild type or mutant α subunits with the auxiliary β1 subunit in human embryonic kidney (HEK 293) cells. *SCN2A* was chosen for this analysis because it expresses extremely well in HEK cells, whereas the cloned rat *SCN1A* did not produce detectable currents when transfected into HEKs or other mammalian cell lines in preliminary experiments. We believe that this methodological approach is valid, since *SCN2A* is also expressed in the brain and shows more than 95% identity to rat *SCN1A* overall, with 100% identity in the vicinity of the mutation (Goldin et al. 2000). Moreover, rat *SCN2A* exhibits electrophysiological properties similar to *SCN1A* when expressed in *Xenopus* oocytes (Smith and Goldin 1998) and mutation in*SCN2A* may also cause epilepsy in humans (Sugawara et al. 2001b). Whole cell current density in HEKs expressing mutant sodium channels was statistically indistinguishable from current

density in cells expressing wild type channels (mean densities at 0mV: wild type: 131 ± 21 pA/pF; D188V: 146 ± 28 pA/pF), indicating that the mutation did not prevent efficient expression of functional channels on the cell surface. For both wild type and mutant channels, the decay of sodium currents was rapid and nearly complete over a range of stimulus potentials (Fig. 3A and B), with a small sustained current at the end of test depolarizations (Fig. 3C). Inactivation time constants (Fig. 3D) and the levels of persistent current (Fig. 3E) were indistinguishable over this entire voltage range. Indeed, the inactivation rates and levels of persistent current in superimposed the means of normalized currents evoked at 0mV, were virtually identical (Fig. 3B and C). These data indicate that the D188Vmutation does not increase neuronal excitability by altering the time course of sodium channel gating. Mean activation (Fig. 4A and B) and inactivation (Fig. 4C) curves for wild type and mutant channels were also virtually identical, indicating that D188V does not change cell excitability by altering the voltage range over which sodium channels open, or by altering the voltage-dependence of sodium channel inactivation. Frequency-dependent cumulative inactivation of sodium currents during high frequency channel activity is thought to play a significant role in shaping normal neuronal electrophysiology (Colbert et al. 1997; Jung et al. 1997), and may also be involved in dampening pathophysiological hyperexcitability associated with seizures. Therefore, we investigated whether the D188V mutation affected the frequency dependence of sodium channel activity by examining wild type and D188V sodium currents during depolarizing trains of pulses at frequencies ranging from 1 to 100 Hz. Interestingly, at frequencies ≥10 Hz, current in cells expressing mutant sodium channels declined in amplitude significantly less than currents in cells expressing wild type

channels (Fig. 5), an effect compatible with an increase in cell excitability. Frequency-dependent cumulative inactivation is caused by incremental decline in the fraction of sodium channels available to open from one depolarizing pulse to the next. Since the main factors determining pulse-to-pulse channel availability are the rate and extent of channel recovery from inactivation between each depolarizing test pulse, we expect wild type and mutant channels to differ in one or both of these parameters. To test this hypothesis, we inactivated channels with an 8ms depolarizing conditioning pulse, and then examined the time course of recovery from inactivation at a hyperpolarized recovery potential. Using this protocol, recovery of both wild type and mutant channels followed a similar exponential time course, reflecting rapid recovery from conventional fast inactivation. Although recovery of wild type and mutant channels was statistically indistinguishable, recovery of mutant channels appeared to be slightly more complete than recovery of wild type channels (Fig. 6A). This small difference was accentuated when we examined recovery using a longer (1 s) conditioning pulse, which roughly approximated the cumulative membrane depolarization during a long train of short test pulses separated by brief recovery intervals. With this longer conditioning pulse, recovery was biexponential, with distinct rapidly and slowly recovering components (Fig. 6B). Two differences between wild type and D188V channels were evident from this analysis. First, the proportion of channels recovering rapidly was larger for D188V (~75%) than for wild type (~60%). Second, the slow component of recovery was faster for mutant channels ($\tau \sim 94$ ms) than for wild type channels ($\tau \sim 190$ ms). Together, these effects resulted in a faster overall recovery rate for the mutant than for wild type. These data suggest that the mutation causes decreased sodium channel entry into and faster recovery

from a distinct slow inactivated state (Rudy 1978), an effect that is barely detectable with short depolarizations but obvious with the long depolarizations. Together, these data suggest that slow inactivated sodium channels accrue gradually during long trains of brief depolarizations. During these high frequency trains, the difference in slow inactivation between wild type and mutant gradually accumulates, resulting in the emergence of a large difference infrequency-dependent cumulative inactivation.

Discussion

In the present study, we describe a D188V mutation in the *SCN1A* gene, in a family that we have previously linked to chromosome 2p36 (GEFS2) (Lopes-Cendes et al. 2000). Several lines of evidence suggest that D188V is the pathogenic mutation in this family: (1) the non-conservative change of an aspartic acid (charged polar) to a valine (uncharged, nonpolar); (2) D188 is conserved in ten different sodium channel alpha subunits found in humans, and also in different sodium channels from invertebrate species, suggesting that this residue is functionally critical (Fig. 2); and (3) the D188V mutation was not found in over 400 control chromosomes. In addition, by introducing the D188V mutation into the highly homologous *SCN2A* sodium channel and comparing the functional properties of wild type and mutant channels expressed in HEK cells, we confirm that the D188V mutation has a functional effect on the sodium currents in vitro, with a phenotype compatible with membrane hyperexcitability. Voltage-gated sodium channels are the dominant excitatory current in excitable membranes and play major roles in determining the action potential threshold and in shaping neuronal firing patterns. In humans, mutations in different sodium channel subtypes have been found to cause

diseases of skeletal muscle (*SCN4A*; hyperkalemic episodic paralysis and paramyotonia congenita) (Cannon and Strittmatter 1993; Chahine et al. 1994), heart (*SCN5A*; long QT syndrome) (Bennett et al. 1995), and brain (*SCNB1*, *SCN1A*, *SCN2A*; GEFS) (Wallace et al. 1998; Escayg et al. 2000b; Sugawara et al. 2001b). In the case of epilepsy, we might expect that subtle changes in sodium channel function could be sufficient to disrupt electrophysiological homeostasis, resulting in seizures. Consistent with this hypothesis, previous studies have shown that the T875M, W1204R and R1648H mutations in *SCN1A* cause small increases in persistent sodium current and other subtle changes in channel gating which may contribute to the GEFS phenotype (Alekov et al. 2000; Alekov et al. 2001; Spampanato et al. 2001; Lossin et al. 2002). In this study, we demonstrate for the first time that the D188V mutation in *SCN1A* also causes a subtle change in channel function, specifically a resistance to cumulative inactivation during high frequency activation, but does not affect persistent current, the time course of inactivation or voltage-dependence of channel gating. This small effect on a single aspect of sodium channel function is consistent with the GEFS+ phenotype, which is characterized by episodes of febrile and afebrile seizures, separated by long seizure-free intervals. Interestingly, two recent studies of other GEFS+ mutations in sodium channel α and $\beta 1$ subunits suggest that reduction in frequency dependent cumulative inactivation may be a common mechanism by which sodium channel mutations cause epilepsy in humans (Spampanato et al. 2001; Meadows et al. 2002). We cannot be certain that the D188V mutation has the same effect in the rat *SCN2A* clone as it would in human *SCN1A* channels. However, rat *SCN2A* is 95% homologous to rat *SCN1A*, is identical in the region surrounding the mutation, and exhibits similar functional properties when

expressed in oocytes. Therefore, it is highly plausible that the mechanism we described here will be the same in human sodium channels, and therefore is relevant to the understanding of this epilepsy syndrome. How does the D188V mutation cause epilepsy? During seizures, individual neurons undergo prolonged membrane depolarizations, during which they continuously fire action potentials at high frequency (McNamara 1994). Cumulative inactivation of sodium currents during this pathological activity would be expected to reduce neuronal excitability and thus dampen neuronal firing. In contrast, neurons expressing sodium channels with the D188V mutation are expected to be more resistant to sustain high frequency firing, and thus more able to sustain and propagate seizure activity in the brain. Although the general idea that decreased sodium current cumulative inactivation will increase neuronal excitability is intuitively obvious, the exact mechanism by which this change in channel function causes seizures in the brain of epileptic patients is less clear. Sodium channels are involved not only in the initiation and propagation of action potentials, but also in synaptic integration (Stuart and Hausser 2001), boosting of synaptic potentials (Stuart and Sakmann 1995) and subthreshold electroresponsiveness (Alonso and Llinas 1989). Sodium channels in different subcellular regions show differing functional properties (Colbert et al. 1997; Jung et al. 1997), and each of the various neuronal sodium channel subtypes exhibits a distinct pattern of regional and subcellular localization (Westenbroek et al. 1989). The protein product of the *SCN1A* gene is localized to the somata of at least some brain neurons (Westenbroek et al. 1989), and thus may play a specialized role in determining neuronal firing patterns. The findings presented here provide a starting point for in vivo

studies to understand the mechanism by which mutations in *SCN1A* lead to epilepsy in humans.

Acknowledgements

The authors wish to thank E. Schurr for chromosome specific PAC clones. P.C. is funded by a Canadian Institutes for Health Research (CIHR) fellowship, clinical-scientist program (FRN-35318). D.S.R. is funded by CIHR grant MT-13485. G.A.R. is supported by the CIHR. This work was supported by Xenon Genetics Inc.

Figures

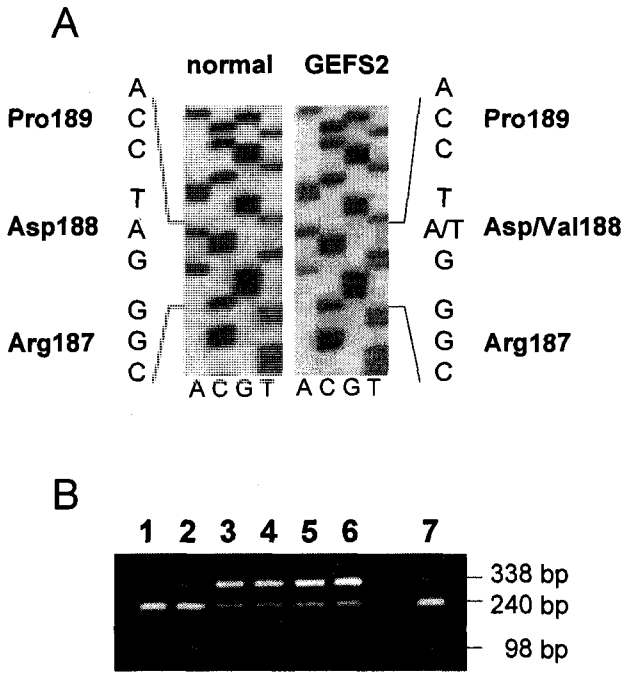


Figure 1. Detection of the D188V mutation. (A) Sequence analysis of PCR products amplified from genomic DNA of a normal control and a GEFS2 patient using NaC-63 and NaC-64 primers. The A-T substitution detected in the heterozygous patient results in a non-conservative amino acid change (D188V). (B) Detection of this mutation using *Bam*HI digestion of the PCR products amplified from four affected patients (lanes 3–6) and three controls (lanes 1–2, and 7) with primers NaC-63 and NaC-64. Normal alleles containing a *Bam*HI site give digested fragments of 240 and 98 bp, whereas the mutant alleles remain undigested at 338 bp.

(A)	SCN1A	F	T	T	L	R	**D**	P	W	N	W	L
	SCN2A	-	-	-	-	-	-	-	-	-	-	-
	SCN3A	-	-	-	-	-	-	-	-	-	-	-
	SCN4A	-	-	-	-	-	-	-	-	-	-	-
	SCN5A	-	-	-	-	-	-	-	-	-	-	-
	SCN6A	-	S	-	-	G	-	-	-	-	-	-
	SCN8A	-	-	-	-	-	-	-	-	-	-	-
	SCN9A	-	-	-	-	-	-	-	-	-	-	-
	SCN10A	-	-	Y	-	-	-	-	-	-	-	-
	SCN11A	-	S	F	-	-	-	-	-	-	-	-
(B)	electric eel	-	-	-	-	-	-	-	-	-	-	-
	puffer fish	-	-	-	-	-	-	-	-	-	-	-
	Aplysia	-	-	Y	-	-	-	-	-	-	-	-
	squid	-	-	Y	-	-	-	A	-	-	-	-
	fruitfly	-	-	Y	-	-	-	A	-	-	-	-
	flatworm	-	-	Y	-	-	S	I	-	-	-	-
	jellyfish	Y	S	Y	-	-	N	S	-	-	-	-

Figure 2. Amino acid sequence alignment of the region near D188 in sodium channel genes. (A) D188 is conserved in the following 10 known sodium channel alpha subunit genes (SCNA) in humans (SwissProt or GenBank accession numbersin parentheses): *SCN1A* (P35498), *SCN2A* (Q99250), *SCN3A* (AAG53415), *SCN4A* (P35499), *SCN5A* (Q14524), *SCN6A* (Q01118), *SCN8A* (AAD20438), *SCN9A* (NP 002968), *SCN10A* (AAD30863), *SCN11A* (NP 054858). (B) D188 is conserved in the majority of non-mammalian species: electric eel (P02719), puffer-fish (BAA90398), Aplysia (AAC47457), squid (AAA16202), fruit-fly (P35500), flatworm (AAC63049), and jellyfish (AAA75572). D188 is shown in bold. Residues that are identical to the *SCN1A* sequence are shown as dashes.

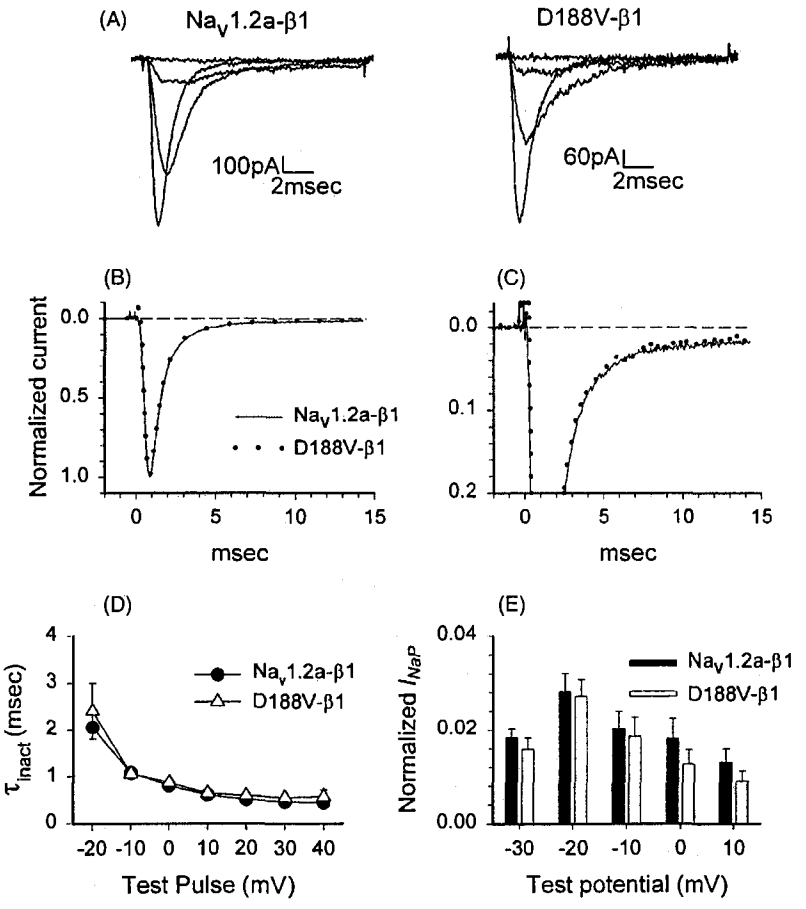

(A)
Na$_V$1.2a-β1
D188V-β1
100pA
2msec
60pA
2msec
(B)
(C)
Normalized current
0.0
0.5
1.0
Na$_V$1.2a-β1
D188V-β1
0
5
10
15
msec
0.0
0.1
0.2
(D)
(E)
τ_{inact} (msec)
Na$_v$1.2a-β1
D188V-β1
-20 -10 0 10 20 30 40
Test Pulse (mV)
Normalized I_{NaP}
0.04
0.02
0.00
-30 -20 -10 0 10
Test potential (mV)

Figure 3. The D188V mutation does not affect the time course or extent of sodium current inactivation. (A) Typical whole cell sodium currents elicited in two different cells one expressing wild type *SCN2A* (Nav1.2a) sodium channel, the other expressing D188V. In this and subsequent figures, wild type and mutant α subunits were coexpressed with wild type β1. Currents were evoked by depolarizations to −35, −25, −15 and −5mV, from a holding voltage of −90mV. (B) Mean whole cell currents, elicited by depolarization to 0mV, for cells expressing wild type (solid trace) or D188V (dotted trace) channels. To obtain these mean traces, wild type or mutant currents evoked at 0mV in each cell were normalized, and then the normalized traces were averaged together. (C) The same traces, rescaled to show the much smaller persistent currents. (D) Mean inactivation time constants for wild type (●, $n = 6$) and D188V (△, $n = 8$) whole cell currents. Whole cell currents evoked over a range of test potentials were fit with a single exponential to determine inactivation time constants. In this and subsequent figures, error bars indicate standard errors of the means. (E) Mean levels of persistent current for wild type ($n = 16$) and D188V ($n = 13$) over a range of test potentials. In each cell, persistent current was measured at the end of depolarizing test pulses, and then normalized with respect to the peak current elicited at 0mV.

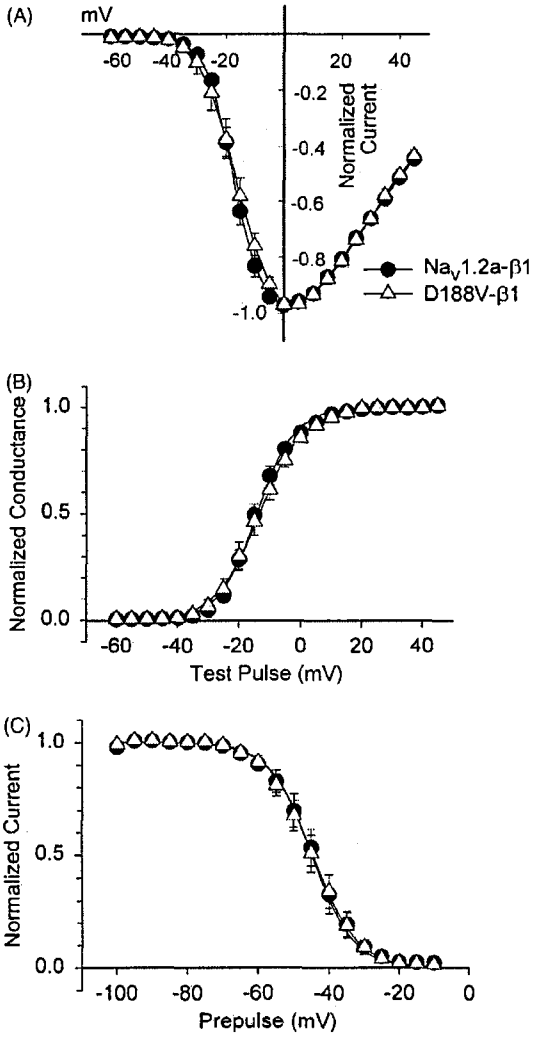

(A)
mV
-60
-40
-20
20
40
-0.2
-0.4
-0.6
-0.8
-1.0
Normalized Current
$Na_V1.2a$-β1
D188V-β1
(B)
Normalized Conductance
1.0
0.5
0.0
-60
-40
-20
0
20
40
Test Pulse (mV)
(C)
Normalized Current
1.0
0.5
0.0
-100
-80
-60
-40
-20
0
Prepulse (mV)

Figure 4. D188V does not alter the voltage-dependence of sodium channel activation or inactivation. (A) Mean, normalized current–voltage relationships for cells expressing wild type (●, $n = 19$) or D188V (△, $n = 14$) channels. The graph shows peak currents, elicited by depolarizations to a range of test potentials from a holding potential of −90mV. (B) Voltage-dependence of activation for wild type and D188V. The current–voltage relationships in panel (A) were converted to activation curves and fit with the Boltzmann equation, as described elsewhere (Hamill et al. 1981; Kazen-Gillespie et al. 2000). Mean values for $V1/2$ and k were as follows: wild type: $V1/2 = -13.9$, $k = -5.4$; D188V: $V1/2 = -13.6$, $k = -5.7$. (C) Voltage-dependence of inactivation for wild type and D188V. Inactivation curves were determined as described in Section 2, from the same cells as in panels (A) and (B), and fit with the Boltzmann equation. Mean values for $V1/2$ and k were as follows: wild type: $V1/2 = -44.5$, $k = 6.3$; D188V: $V1/2 = -44.8$, $k = 6.0$.

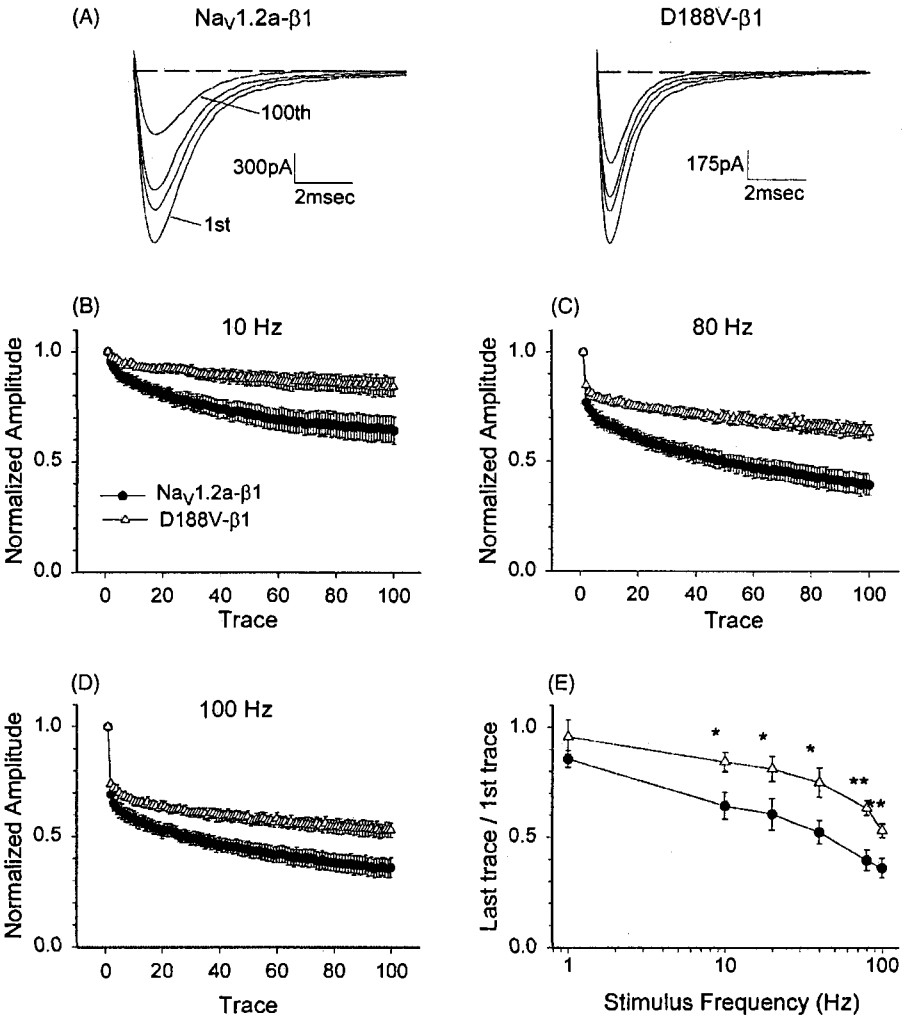

(A)
Na$_V$1.2a-β1
D188V-β1
100th
1st
300pA
2msec
175pA
2msec
(B)
10 Hz
(C)
80 Hz
Normalized Amplitude
Na$_V$1.2a-β1
D188V-β1
Trace
(D)
100 Hz
(E)
Last trace / 1st trace
Stimulus Frequency (Hz)

Figure 5. D188V reduces cumulative inactivation of sodium current amplitude during high frequency trains of channel activation. (A) Typical current traces elicited by the 1st, 2nd, 10th and 100th pulses of 80 Hz trains in cells expressing wild type (left hand traces) or D188V(right hand traces) channels. Each trace was evoked by 5 ms depolarization to 0mV, from a holding voltage of −80mV. (B–D) Mean, normalized amplitudes of currents elicited by pulse trains of 10 (B), 80 (C) or 100 (D) Hz, in cells expressing wild type (●) or D188V(△) channels. (E) The extent of frequency dependent cumulative inactivation for wild type and D188V channels over a range of stimulus frequencies. The data points show means of amplitudes of the last pulse in the train/the 1st pulse in the train. Single and double asterisks indicate statistically significant ($P < 0.05$) and highly significant ($P < 0.01$) differences between wild type and D188V, respectively. Means were determined from 3 (1 Hz) or 10 (10–100 Hz) experiments, respectively.

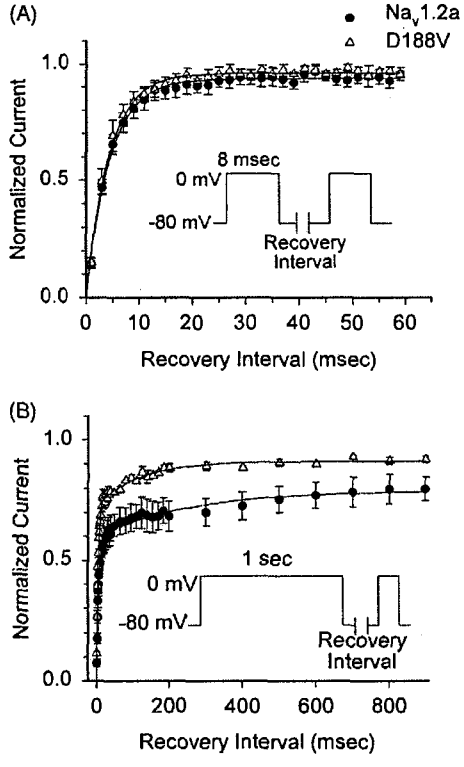

(A)
$Na_v1.2a$
D188V
Normalized Current
1.0
0.5
0.0
8 msec
0 mV
-80 mV
Recovery Interval
0 10 20 30 40 50 60
Recovery Interval (msec)
(B)
Normalized Current
1.0
0.5
0.0
1 sec
0 mV
-80 mV
Recovery Interval
0 200 400 600 800
Recovery Interval (msec)

Figure 6. D188V reduces slow inactivation. (A) Mean recovery from inactivation, for cells expressing wild type (●, $n = 4$) or D188V (△, $n = 5$) channels. Recovery time course was determined, using a standard three-step protocol, as described previously (Hamill et al. 1981; Kazen-Gillespie et al. 2000) (see inset). Channels were inactivated by an 8ms conditioning pulses to 0mV. This was followed by a recovery interval of varying duration at −80mV and then a test pulse to 0mV to assess the fraction of recovered channels. The graph shows the amplitude of the currents evoked by the test pulse, normalized with respect to complete recovery, and plotted as a function of the recovery interval. The smooth lines are exponential fits. Mean recovery times constants were 2.9±0.2 and 2.9±0.4, for wild type and D188V, respectively. (B) Mean recovery from inactivation, determined using 1 s conditioning pulses to 0mV (see inset). The data were fit with the sum of two exponentials, with mean time constants of 4.9±1.1 and 186±4.8 for wild type ($n = 4$) and 3.6 ± 0.2 and 94.0 ± 32.1 for D188V ($n = 4$), respectively.

CHAPTER 3: MUTATION OF GABRA1 IN AN AUTOSOMAL DOMINANT FORM OF JUVENILE MYOCLONIC EPILEPSY

Reference :

Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, and Rouleau GA. Mutation of *GABRA1* in an autosomal dominant form of Juvenile Myoclonic Epilepsy. *Nature Genetics* 2002; 31: 184-189.

Preface

"I am about to discuss the disease called "sacred." It is not, in my opinion, any more divine or more sacred than any other diseases, but has a natural cause ...Its origin, like that of other diseases, lies in heredity... My own view is that those who first attributed a sacred character to this malady were like the magicians, purifiers, charlatans, and quacks of our own day..."
HIPPOCRATES
470-410 BC

Even in modern classification, tonic-clonic seizures are reffered to *Grand Mal* epilepsy, as if the individual could be *possessed.* This surprising appellation probably reflects a combination of mystery about etiology of the disease, together with astonishment caused by its spectacular clinical manifestation. Genetics factors have been suspected for many years to play an important role in the etiology of idiopathic generalized epilepsy (IGE). However, despite the recent identification of gene defects in families segregating rare epileptic syndromes, genes underlying the classical IGE remained to be determined. In this chapter, the identification of the first gene predisposing to juvenile myoclonic epilepsy, one of the most common IGE syndromes, is described. In this family, a missense mutation in the α1 subunit of the $GABA_A$ receptor (*GABRA1*) was found to cause a loss-of-function of this ligand-gated chloride channel. Meanwhile, genetic studies of large pedigrees segregating other classical IGE phenotypes led to the identification of mutations in the γ2 (*GABRG2*) and the δ (*GABRD*) subunit of the $GABA_A$ receptor, as well as in the voltage-gated chloride channel (*CLCN2*), suggesting that these syndromes may be caused by complementary molecular mechanisms. Another mutation in *GABRA1* was also found recently, in collaboration (see appendix 2).

Abstract

Although many genes that predispose for epilepsy in humans have been determined, those that underlie the classical syndromes of idiopathic generalized epilepsy (IGE) have yet to be identified. We report that an Ala322Asp mutation in *GABRA1*, encoding the α1 subunit of the γ-aminobutyric acid receptor subtype A ($GABA_A$), is found in affected individuals of a large French Canadian family with juvenile myoclonic epilepsy. Compared with wild type receptors, $GABA_A$ receptors that contain the mutant subunit show a lesser amplitude of GABA-activated currents *in vitro*, indicating that seizures may result from loss of function of this inhibitory ligand-gated channel. Our results confirm that mutation of *GABRA1* predisposes towards a common idiopathic generalized epilepsy syndrome in humans.

Introduction

Idiopathic generalized epilepsy is a common neurological disorder that affects roughly 0.4% of the population. The disease has a significant hereditary component, as indicated by studies of family aggregation and twins (Annegers et al. 1982; Berkovic et al. 1998). But the identification of genes that underlie predisposition to IGE is hampered by clinical heterogeneity of the syndromes and by genetic heterogeneity for apparently similar syndromes. Clinical studies of large kindreds have allowed the definition of a new IGE syndrome that is associated with febrile seizures (generalized epilepsy with febrile seizures, GEFS) (Scheffer and Berkovic 1997). At least three genes, which encode the β1 and α1 subunits of the neuronal voltage-gated sodium channel (*SCN1B*, *SCN1A*)

(Wallace et al. 1998; Escayg et al. 2000b; Wallace et al. 2001b) and the 2 subunit of $GABA_A$ receptor (*GABRG2*) (Baulac et al. 2001; Wallace et al. 2001a) have been found to underlie GEFS. Only a few families affected with GEFS, however, carry mutations in these genes. In addition, the proportion of individuals with IGE that meet the criteria for GEFS is currently unknown; thus, the genes critical to most types of sporadic and familial IGE have yet to be determined.

Results

We collected and examined DNA from 14 members of a French Canadian family with epilepsy (Fig. 1). All affected family members have a similar phenotype (Table 1) and fulfill the criteria for juvenile myoclonic epilepsy (JME) (Genton et al. 1994; Janz and Durner 1997). The only exception is individual IV-03, who has an earlier onset of disease but has clinical features that are otherwise indistinguishable from other members of the family (Fig. 2 and Table 1). This observation is consistent with previous reports of early-onset JME syndrome (Wolf 1992; Janz and Durner 1997) and reinforces the concept of a biological continuum among classical IGE syndromes (Berkovic et al. 1987). We did not find any history of febrile seizure in this family. By systematically recording electroencephalograms (EEGs) during childhood in every member of this large kindred, we found a photoparoxysmal response (photosensitivity) in individual IV-02, although he denied having any symptoms suggestive of a seizure disorder. As a photoparoxysmal response is found in up to 8.3% of children (Eeg-Olofsson et al. 1971; Doose and Gerken 1973) we did not consider him to be affected for the purpose of this linkage study.

We conducted a genome scan using 383 evenly distributed microsatellite markers. We found evidence of linkage to chromosome 5q34, with a maximum lod score of 3.1 at =0 for marker D5S414. This region represents the only lod score above 2.0 in the whole genome. Fine-mapping shows that the candidate region includes a cluster of $GABA_A$ receptor subunits—β2 (*GABRB2*), α1 (*GABRA1*) and γ2 (*GABRG2*)—that are flanked by the markers D5S1955 and D5S422 (Fig. 1). We screened the family for mutation in these GABA receptor genes using both single-stranded conformation polymorphism (SSCP) analysis and denaturing high-performance liquid chromatography (dHPLC). We did not find any mutations in *GABRG2*; however, we detected a variant in exon 9 of *GABRA1* in all of the affected (n = 8), but none of the unaffected (n = 6), members of the family. We did not find mutations in the other exons of *GABRA1*.

Sequence analysis indicated that the affected individuals are heterozygous with respect to a C→A substitution, which is predicted to change a GCC (alanine) to a GAC (aspartic acid) codon at position 322 of the *GABRA1* cDNA (Fig. 3a). This alanine residue is located in the third transmembrane domain of the predicted protein and is conserved in all α-subunits of $GABA_A$ receptors of different species (Fig. 3b,c). We did not find this Ala322Asp variation in 400 control chromosomes of individuals of French Canadian origin, nor did we find it in individual IV-02, who showed only an abnormal EEG. In addition, we did not find the Ala322Asp mutation in people with sporadic IGE, which included individuals with JME (n = 31) and childhood absence epilepsy (CAE) syndromes (n = 52). Some of the individuals with sporadic IGE had a positive family history for various epileptic syndromes (5 affected with JME and 16 affected with CAE), but these families were not large enough to test for linkage.

We investigated whether the Ala322Asp mutation altered the functional properties of $GABA_A$ receptors in vitro. $GABA_A$ receptors are ligand-gated chloride channels that mediate fast synaptic inhibition in the brain. Their molecular structure comprises a heteropentameric protein complex, which is assembled from different classes of subunits (α1–6, β1–4, γ1–3, δ, ε, π, θ) (Moss and Smart 2001). The $\alpha1\beta2\gamma2$ combination is thought to be the most abundant form of the receptor in the brain (Sieghart et al. 1999). The optimal subunit stoichiometry and composition required for functional expression of $GABA_A$ receptors with the full pharmacological spectrum of the native receptors is a 2:2:1 ratio of α, β and γ subunits. We therefore cloned *GABRG2*, *GABRA1* and *GABRB2* cDNA and introduced the Ala322Asp mutation into the *GABRA1* cDNA. HEK 293 cells expressing mutant $GABA_A$ receptor (α1Ala322Asp22) have a lower amplitude of GABA-activated currents than those expressing the wildtype ($\alpha1\beta2\gamma2$) receptor (Fig. 4a,b).

We also introduced the mutation into rat *GABRA1* cDNA, which caused a similarly lesser amplitude of GABA currents gated through mutant receptors. Immunoblot analysis using an antibody specifically directed against the α1 subunit of the $GABA_A$ receptor did not indicate a difference in the amount of expression between mutant and wildtype subunits (Fig. 4c). Thus, the lesser amplitude of the GABA currents observed in mutant $GABA_A$ receptors is not due to a reduced amount of receptor expression but instead results from an alteration in their function.

To determine further the mechanisms underlying these functional alterations, we analyzed the dose-response relationship of GABA-evoked currents (Fig. 5). As expected, the maximum response to GABA was lower in cells that expressed mutant

α1Ala322Asp22 receptors. This result suggests that the Ala322Asp mutation might affect the channel gating properties, for example, by reducing the single channel conductance and open probability. Alternatively, the amount of receptors expressed at the cell surface might be reduced. There was a clear shift in the dose–response curve toward the right, which shows that the ligand-binding affinity of the mutant is reduced markedly compared with that of the wild type. Finally, diazepam significantly potentiated GABA currents of both wildtype and mutant channels, to a similar extent (data not shown).

In cells expressing either the mutant or the wildtype $GABA_A$ receptor, the same phenotype was observed consistently, without overlap in the relative amplitude of the GABA-evoked currents (Figs 4 and 5). Because the disease is an autosomal dominant trait, we attempted to assess in vitro the functional impact of the Ala322Asp mutation in a 'heterozygous' condition by transfecting an equal amount of mutant and wildtype *GABRA1* cDNA. In a preliminary study, none of the recorded cells showed an obvious intermediate response; that is, some cells presented a 'mutant phenotype', whereas others showed a 'wildtype phenotype', as though one subunit (either mutant or wildtype α1) is incorporated preferably into the heteropentameric protein complex. These data must be interpreted with caution, however, because it is virtually impossible to control adequately for differences in protein expression. In addition, we can make only limited inferences in regard to the functional consequences of the mutant GABA subunit *in vivo*, because there are at least 17 different GABA subunits available in the brain for assembly into a fully functional $GABA_A$ receptor.

Discussion

Juvenile myoclonic epilepsy is a common epileptic syndrome affecting up to 26% of all individuals with IGE (Genton et al. 1994; Janz and Durner 1997). Despite much clinical evidence to incriminate hereditary factors in JME, the mode of inheritance of this syndrome remains debated (Panayiotopoulos and Obeid 1989; Wolf 1992), and linkage studies have yielded conflicting results (Greenberg et al. 1988; Weissbecker et al. 1991; Elmslie et al. 1996; Elmslie et al. 1997; Greenberg et al. 2000). These discrepancies probably reflect the fact that JME, as well as other related IGE syndromes, is not usually inherited in a clearly Mendelian manner but instead shows a complex pattern of inheritance. In the family studied here, the transmission is clearly autosomal dominant, with people affected over four generations. Notably, however, the clinical features are homogeneous in all affected members. This contrasts with previous reports of large kindreds affected with either generalized or partial epilepsy, for which the clinical phenotype is highly variable (Scheffer and Berkovic 1997; Picard et al. 2000). In GEFS syndrome, for example, affected individuals present clinical features ranging from febrile seizures alone to a very severe form of epilepsy that is associated with mental retardation (Scheffer and Berkovic 1997; Wallace et al. 1998; Escayg et al. 2000b; Wallace et al. 2001b). For each of the 'IGE genes' identified so far, the common clinical manifestation in mutation carriers is not epilepsy *per se*, but febrile seizure (Scheffer and Berkovic 1997; Wallace et al. 1998; Escayg et al. 2000b; Baulac et al. 2001; Wallace et al. 2001a; Wallace et al. 2001b). The mutation in *GABRA1* is therefore the first example of a mutation that segregates with a common IGE syndrome.

Juvenile myoclonic epilepsy and GEFS syndromes differ significantly in clinical features: individuals who have JME have only afebrile seizures, onset in adolescence

(rather than childhood) and myoclonic jerks (which are usually absent in GEFS syndrome). When expressed in vitro, however, both the Ala322Asp mutation in *GABRA1* and a Lys289Met mutation in *GABRG2*, which has been identified in a family affected with GEFS (Baulac et al. 2001), produce the same electrophysiological effect—that is, a reduction in GABA-activated currents. Differences in the developmental features, or in the relative anatomical distribution of the γ2 and α1 subunits, might explain some of the differences observed in the clinical expression of the disease (Pirker et al. 2000). *In vivo* studies of mutated *GABRA1* and *GABRG2* subunits will help to address these issues and will allow the design of specifically targeted drugs to treat this chronic and disabling disease.

Methods

Genotyping and linkage analysis. We first conducted genotyping at the Genome Centre of the Montreal General Hospital, by using a modified Multi-Probe II (Packard) and ABI 3700 DNA sequencer. The microsatellite markers were obtained from the Whitehead Institute as a modified version of the Cooperative Human Linkage Center screening set and comprised 383 polymorphic, fluorescently labeled markers covering the whole genome with an average intermarker spacing of 10 cM. The marker map positions were based on sex-averaged maps from Marshfield Medical Research Foundation. We used PEDMANAGER software for allele binning and inheritance analysis. Subsequent markers used for the fine-mapping were genotyped manually by PCR incorporating [35S]dATP. We analyzed PCR products by electrophoresis on 6% denaturing polyacrylamide gels, followed by autoradiography. Allele sizes for each marker were obtained from the Fondation Jean Dausset CEPH database and determined by comparison with the M13mp18 sequence ladder. We calculated two-point lod scores using the M-link program (Lathrop and Lalouel 1984; Lathrop et al. 1984) assuming autosomal dominant inheritance with 90% penetrance, a gene frequency of 0.001 and the marker allele frequencies contained in the CEPH database.

Mutation screening. We determined the genomic organization of human *GABRA1* by aligning the sequence of *GABRA1* mRNA with the corresponding genomic sequence on chromosome 5 (Venter et al. 2001); both sequences were obtained from databases. Primers were designed to amplify fragments of 150–300 bp from genomic DNA to screen all coding portions of the gene. We amplified portions of *GABRA1* by PCR and analyzed

them by SSCP and dHPLC (Xiao and Oefner 2001). We used primers GAB-91 and GAB-92 to amplify exon 9 (primer sequences are available upon request). PCR products that showed a conformational change and/or differences in their dHPLC retention pattern were re-amplified from genomic DNA and sequenced using the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB).

Cloning of GABAA receptor subunits and mutagenesis. We designed primers to amplify the complete open reading frame of three different $GABA_A$ subunits ($\alpha1$,$\beta2$,$\gamma2$) from a cDNA fetal brain library (Clontech) by PCR using Pfu polymerase (Stratagene). We cloned the appropriate PCR products into the Topo 2.1 vector (Invitrogene) and subcloned them into the pcDNA3.1(-) vector (Invitrogene). We used the QuickChange site-directed mutagenesis kit (Stratagene) to introduce the Ala322Asp mutation in the *GABRA1* clone using primers GAB-103 and GAB-104. For the analysis of rat $GABA_A$ receptors, we used the previously described $\alpha1$, $\beta2$ and $\gamma2$ subunits (Wan et al. 1997). We introduced the Ala322Asp mutation into the rat *GABRA1* clone using primers RGA-1 and RGA-2. All constructs were sequenced completely to confirm the mutation and to exclude any other variants that might have been introduced during PCR amplification. Primer sequences are available upon request.

Electrophysiological studies of the Ala322Asp mutation. We transfected cDNA encoding wildtype and Ala322Asp *GABRA1* subunits into HEK 293 cells along with cDNAs encoding the *GABRB2* and *GABRG2* subunits. Expressed heteropentameric channels were examined using whole-cell recording techniques in voltage-clamp mode. The details

of HEK cells preparation, cDNA transfection and recordings have been described (Wan et al. 1997). HEK 293 cells were plated on 22-mm glass coverslips coated with poly-d-lysine and transfected with 4 μg of α1, 4 μg of β2 and 2 μg of γ2 $GABA_A$ receptor subunit plasmids, as well as 2 μg of GFP plasmid as a marker of tranfectants, using Lipofectamine (Gibco) according to the manufacturer's protocol. We obtained recordings of GABA-evoked currents 24-48 h after transfection. Whole-cell recordings were made using an Axopatch-1D amplifier (Axon Instruments) at a holding potential of -60 mV. The recording chamber was perfused at room temperature with an extracellular solution containing 140 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl2, 2 mM MgCl2, 10 mM HEPES and 25 mM glucose (pH 7.4, 310–320 mOsm). Recording pipettes (4–5 MΩ) were filled with intracellular solution containing 135 mM CsCl, 2 mM MgCl2, 0.1 mM CaCl2, 0.5 mM EGTA, 10 mM HEPES and 4 mM K+-ATP (pH 7.2, 300–310 mOsm). We applied GABA (50 μM, 100 ms) through a drug pipette close to the recorded cell, except in experiments to establish the dose-response curve, in which different concentrations of GABA were applied to the cells through a computer-controlled multibarreled perfusion system (Warner Instruments), as described (Lu et al. 1998). Because the differences between wildtype and mutant $GABA_A$ receptors were so marked, it was not necessary to make recordings by an investigator who was blind to which *GABRA1* cDNA (mutant or wildtype) had been transfected.

Immunoblot analysis. HEK 293 cells were collected 24 h after transfection and sonicated on ice in a lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1% Nonidet-P40, 0.5% SDS, 1 mM EDTA, 10 g ml-1 aprotinin, 10 g ml-1

leupeptin and 2 mM phenylmethylsulfonyl fluoride. After 10 min of centrifugation at 10,000g, the protein concentration in the supernatant was measured using a bicinchroninic acid protein assay kit (Bio-Rad). A total of 30 μg protein for each transfection group was loaded in SDS sample buffer, separated on a 10% SDS–PAGE gel and then transferred to a nitrocellulose membrane. The blot was probed with a polyclonal antibody against $GABA_A$ α1 (1:1,000; Upstate) and a horseradish peroxidase–conjugated goat antibody against rabbit IgG (1:5,000), and developed using the ECL western blot detection system (Amersham).

Accession numbers. *GABRA1* mRNA, GenBank NM_000806; corresponding genomic sequence on chromosome 5, Celera GA_x2HTBL3TTD1:500001-1000000; mouse *GABRA1*, NP_034380.1; bovine *GABRA1*, A27142; chicken *GABRA1*, P19150; human *GABRA2*, NP_000798.1; human *GABRA3*, NP_000799.1; human *GABRA4*, NP_000800.1; human *GABRA5*, NP_000801.1; human *GABRA6*, NP_000802.1.

URLs. The PEDMANAGER program can be found at: http://www-genome.wi.mit.edu/ftp/distribution/software/pedmanager/.

Acknowledgements

We thank the family for participating in this study, and G. Geoffroy, who brought them to our attention. We also thank W. Ju for assistance in cell transfection experiments, and C. Darmond-Zwaig and T. J. Hudson from the Genome Centre of the Montreal General Hospital. P.C. is funded by a fellowship from the Canadian Institute for Health Research (CIHR), under the clinical-scientist program. G.A.R. is supported by the CIHR.

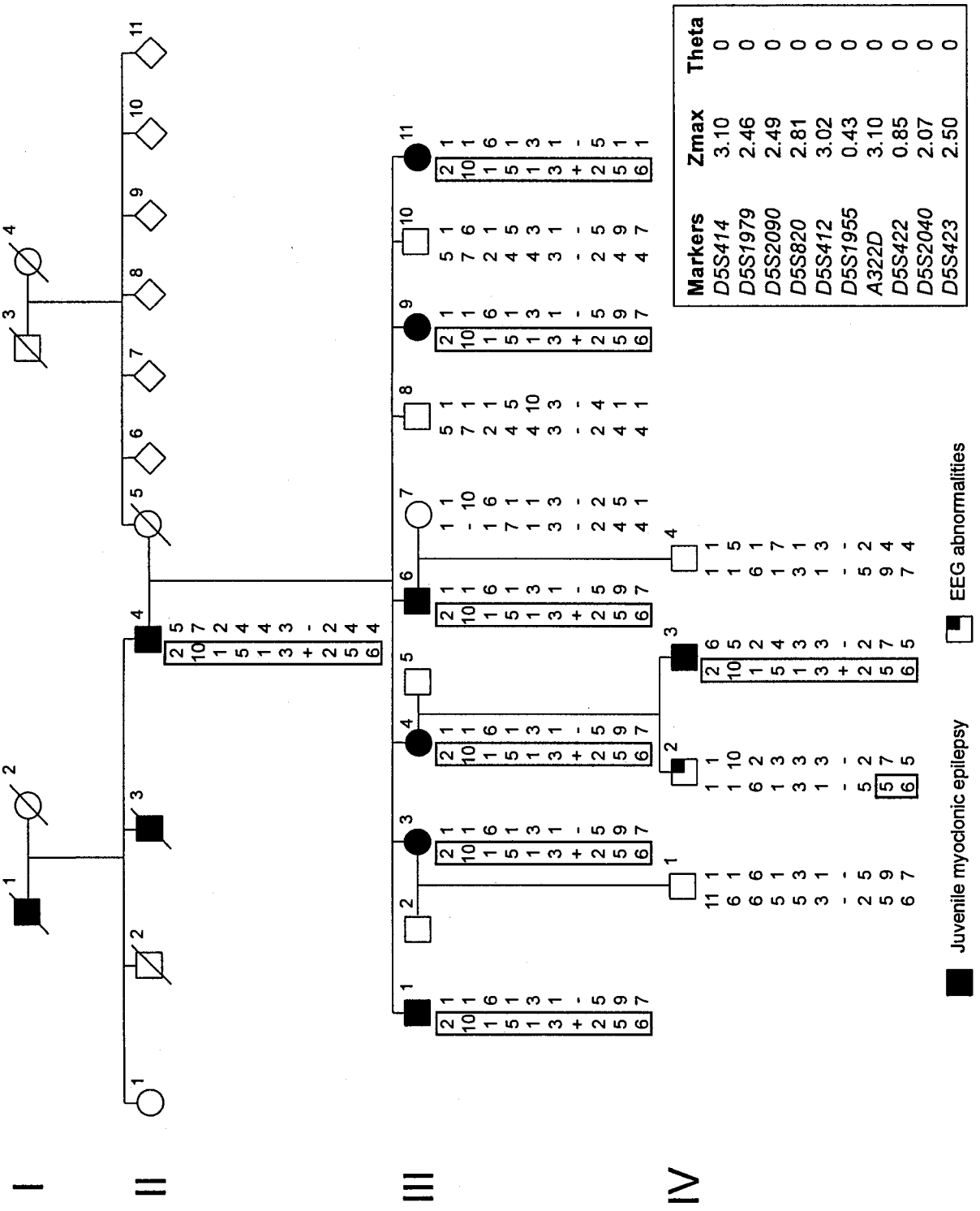

I
II
III
IV
Markers Zmax Theta
D5S414 3.10 0
D5S1979 2.46 0
D5S2090 2.49 0
D5S820 2.81 0
D5S412 3.02 0
D5S1955 0.43 0
A322D 3.10 0
D5S422 0.85 0
D5S2040 2.07 0
D5S423 2.50 0
Juvenile myoclonic epilepsy
EEG abnormalities

Figure 1. A large French Canadian kindred affected with JME.

The clinical features of the epileptic syndrome are homogeneous in all affected members of the family over four generations. We assessed individual I-01 and II-03 indirectly via individual II-04. The phenotype of individual I-01 and II-03 seems to be compatible with JME. Individual IV-02 has EEG abnormalities (photosensitivity), but does not present clinical seizures. The disease-associated haplotype is boxed. Alleles were numbered according to their respective size and corresponding to the CEPH genotyping database. The maximum lod scores obtained with these markers are shown on the lower right. Individuals II-04, III-07 and IV-03 are homozygous for markers *D5S1955* and *D5S422*; this explains why these markers, which otherwise flank the mutated copy of *GABRA1* (+), show lower lod scores than the other adjacent markers on the same haplotype.

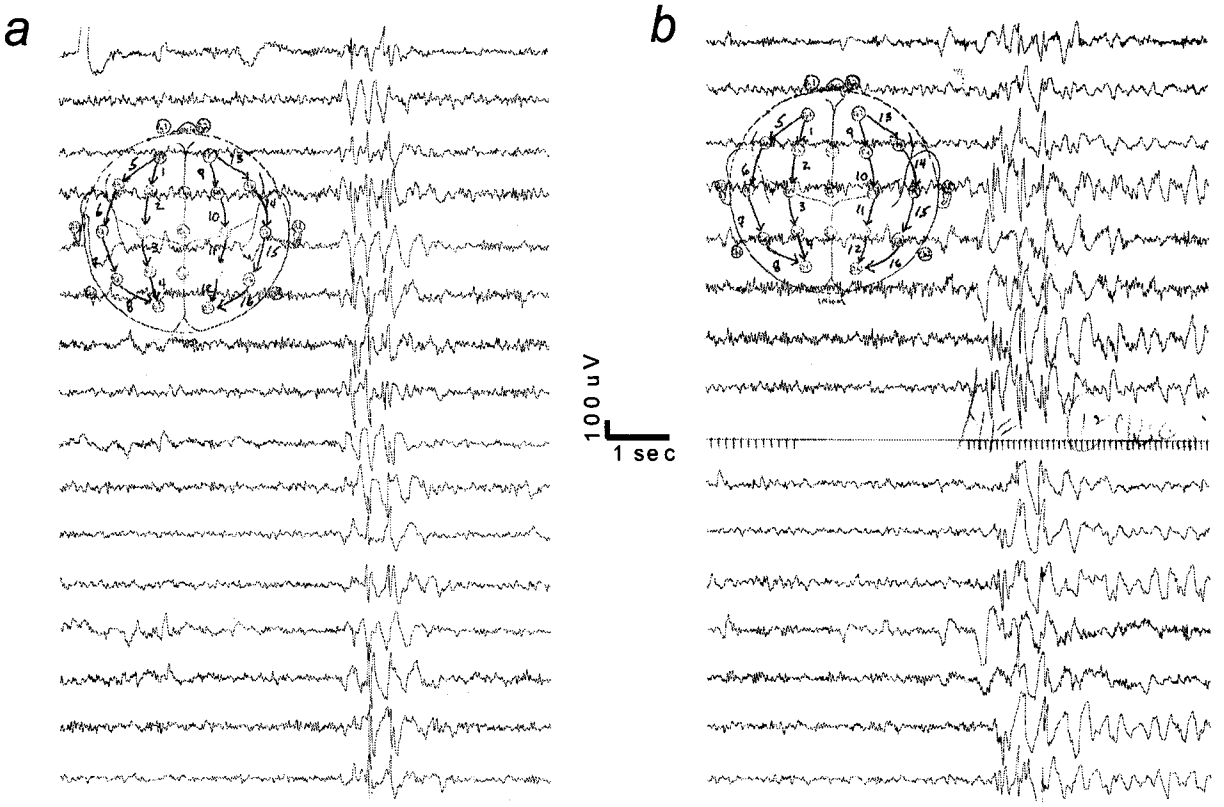
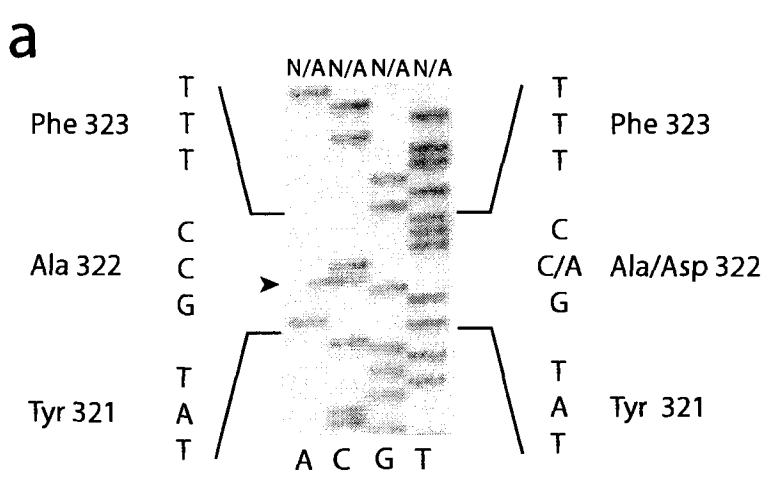
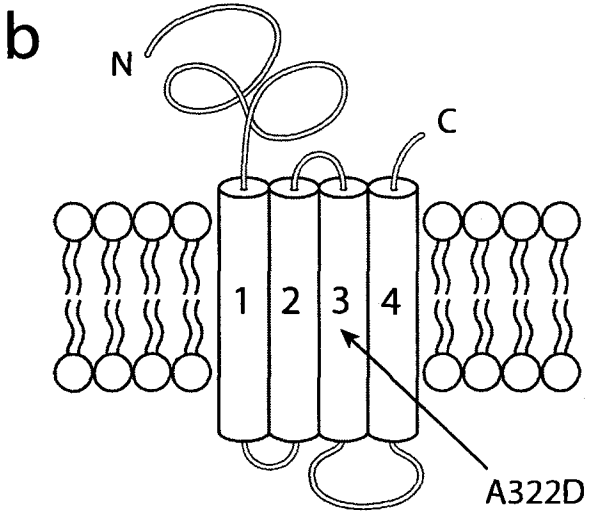


Figure 2. Electroencephalogram abnormalities found in affected members of the family. a,b, All affected members of the family show fast (>3.5 Hz) generalized polyspike-and-wave epileptic discharges, which can occur spontaneously (a) or can be precipitated by photic stimulation (b). These EEG recordings were obtained from individual IV-03.





C

M2 M3

```
human   GABRA1   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDG
bovine  GABRA1   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDG
mouse   GABRA1   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDG
chicken GABRA1   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYAWDG

human   GABRA2   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGWTWDG
human   GABRA3   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRSWAWEG
human   GABRA4   INKESVPARTVFGITTVLTMTTLSISARHSLPKVSYLTAMDWFIAVCFAFVFSALIEFAAVNYFT--------
human   GABRA5   LNRESVPARTVFGVTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGWAWDG
human   GABRA6   INKESVPARTVFGITTVLTMTTLSISARHSLPKVSYATAMDWFIAVCFAFVFSALIEFAAVNYFT--------
                 * *         *              *    * *         *             *     ********
```

Figure 3. The Ala322Asp mutation in *GABRA1* is located in a highly conserved domain of the protein. ***a***, Detection of the Ala322Asp mutation. Sequence analysis of PCR products amplified from genomic DNA. For a given nucleotide (A, C, G, T), each lane in the sequencing gel includes a normal control (N, left) and an affected member of the family (A, right). The affected individual is heterozygous for a C→A substitution (arrowhead), which results in a nonconservative change in aa 322 of the predicted protein (Ala322Asp). ***b***, Location of the mutated residue in the predicted protein. Ala322 is located in the middle of the third transmembrane domain of the predicted protein (arrow). ***c***, Amino-acid sequence alignment of the region near Ala322 in the α1 subunit genes. This alanine (boxed) is conserved in all $GABA_A$ α-subunit genes from different species; mouse, bovine and chicken are included here as examples. Ala322 is also conserved in the six known human $GABA_A$ α-subunit genes: *GABRA2*, *GABRA3*, *GABRA4*, *GABRA5* and *GABRA6*. The residues of the second and third transmembrane domains (M2, M3) are highly conserved among the various $GABA_A$ α-subunits. Nonconserved residues are indicated by an asterisk.

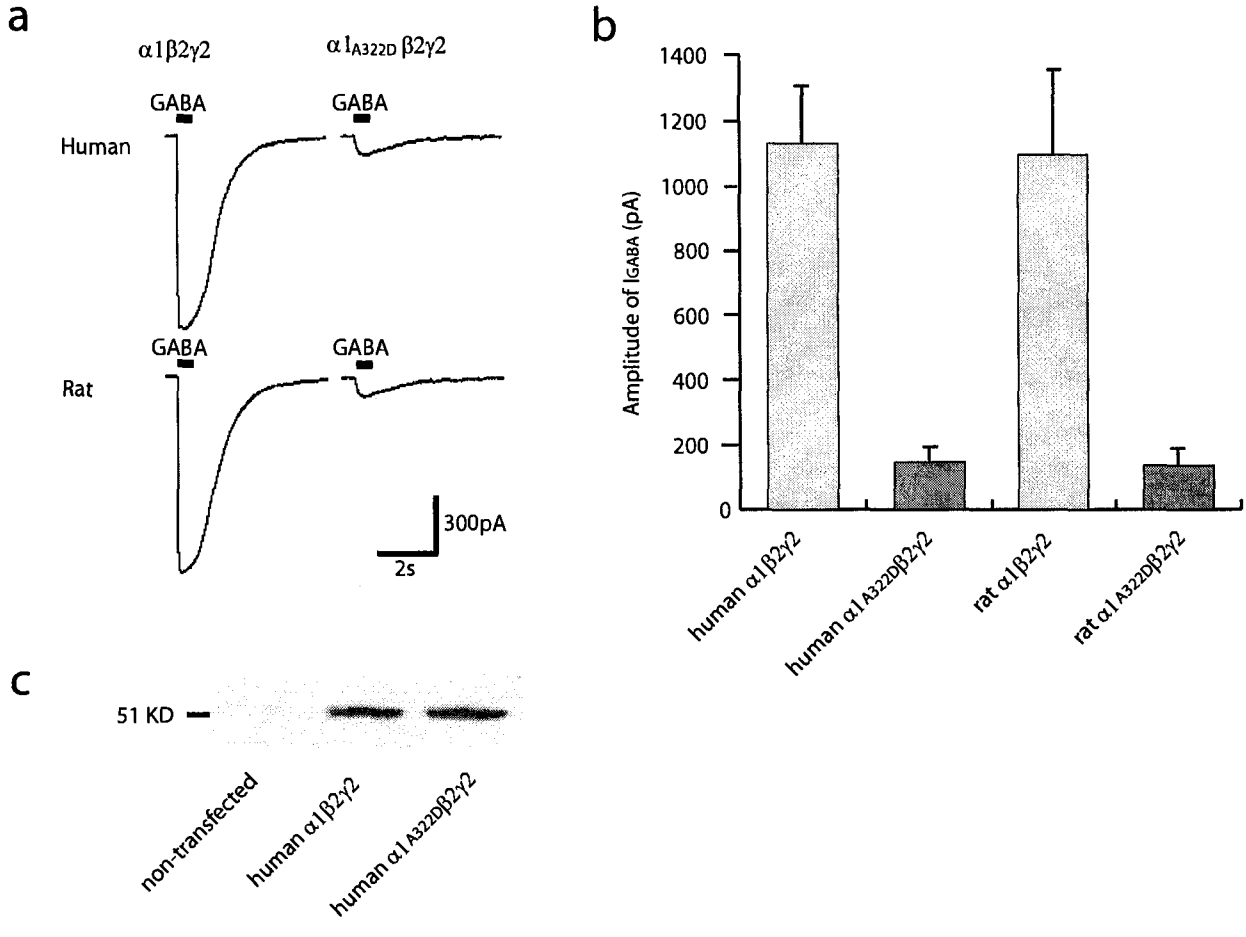

a
α1β2γ2
α1A322D β2γ2
GABA
GABA
Human
GABA
GABA
Rat
300pA
2s
b
Amplitude of IGABA (pA)
1400
1200
1000
800
600
400
200
0
human α1β2γ2
human α1A322Dβ2γ2
rat α1β2γ2
rat α1A322Dβ2γ2
c
51 KD
non-transfected
human α1β2γ2
human α1A322Dβ2γ2

Figure 4. Effect of the Ala322Asp mutation on GABA-evoked currents in transfected HEK 293 cells. ***a***, Representative individual recording trace of whole-cell currents induced by puffing GABA (50 mM, 100 ms) onto the transfected cells at a holding potential of -60 mV. ***b***, Average peak amplitude of the GABA-evoked whole-cell currents from cells transfected with wildtype or mutated *GABRA1*. ***c***, Immunoblot analysis shows that the Ala322Asp mutation does not alter expression of the α1 subunit. Cell lysates from HEK 293 cells transfected with human wildtype (α1β2γ2) or mutant ($\alpha1_{Ala322Asp}\beta2\gamma2$) receptors and from nontransfected cells were immunoblotted with an antibody against the α1 subunit of the $GABA_A$ receptor.

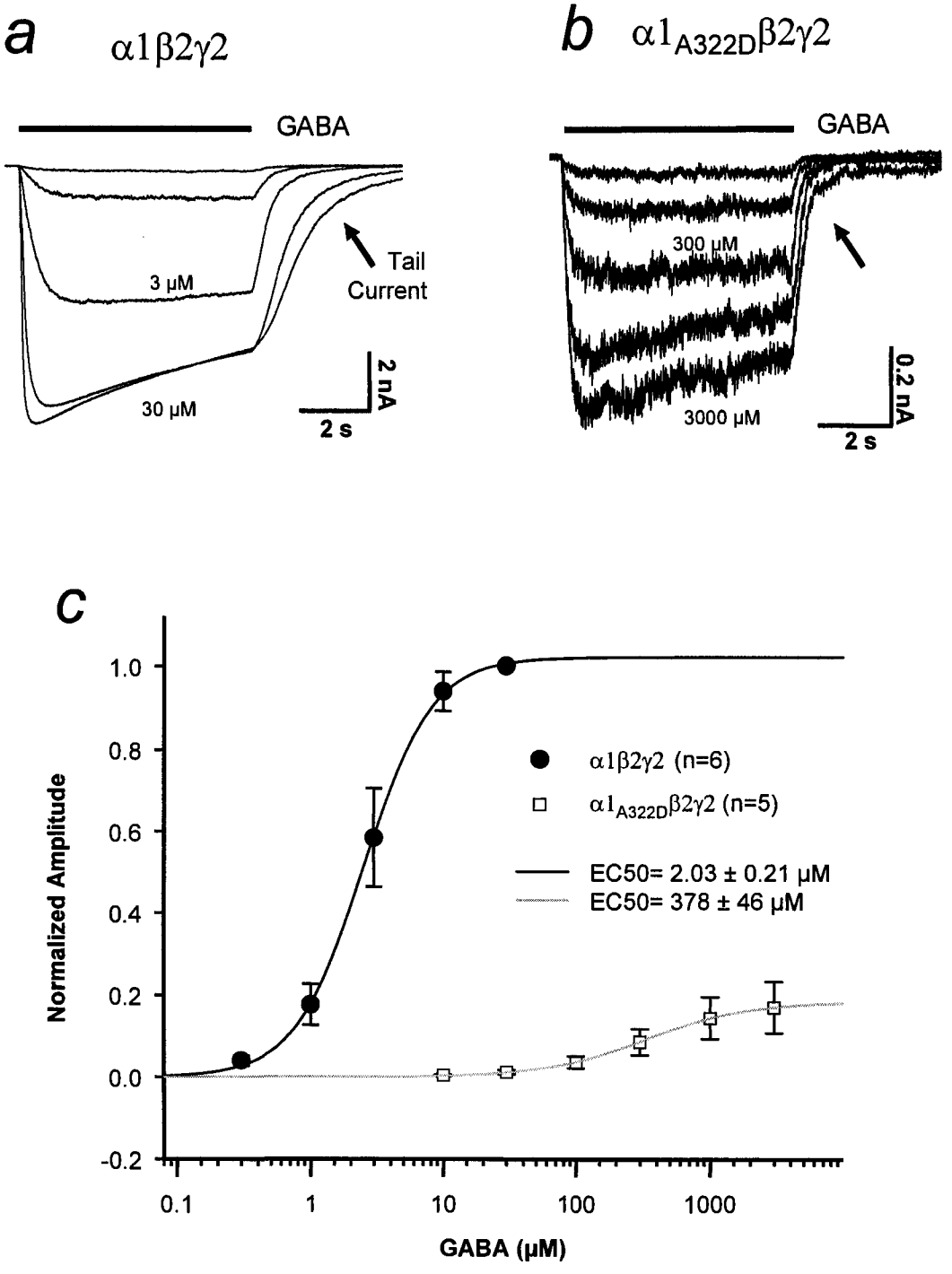

a
α1β2γ2
GABA
3 μM
30 μM
Tail
Current
2 nA
2 s
b
α1A322Dβ2γ2
GABA
300 μM
3000 μM
0.2 nA
2 s
c
Normalized Amplitude
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.1
1
10
100
1000
GABA (μM)
α1β2γ2 (n=6)
α1A322Dβ2γ2 (n=5)
EC50= 2.03 ± 0.21 μM
EC50= 378 ± 46 μM

Figure 5. Effect of the Ala322Asp mutation on the maximum response and affinity of the $GABA_A$ receptors. Whole-cell recordings were made in HEK 293 cells expressing either human $\alpha1\beta2\gamma2$ ($n = 6$) or $\alpha1_{Ala322Asp}\beta2\gamma2$ (n = 5) receptors at a holding membrane potential of -60 mV. Currents were evoked by fast perfusions of GABA at different concentrations through a multibarreled fast perfusion system. ***a***, Examples of individual current traces at different concentrations. Note that different scales were used to record from human wildtype ($\alpha1\beta2\gamma2$) and mutant ($\alpha1_{Ala322Asp}\beta2\gamma2$) receptors. ***b***, Dose-response curves. The peak of individual GABA-induced currents from both the wildtype ($\alpha1\beta2\gamma2$) and mutant ($\alpha1_{Ala322Asp}\beta2\gamma2$) receptors was normalized to the maximal response from the wildtype receptors and plotted against the concentration of GABA. The concentration-response curves were fitted to the Hill equation, and their EC50s (concentration of a drug that produces 50% of its maximal effect) were determined. The Ala322Asp mutation results in both a lowered maximum response and a lesser affinity.

CHAPTER 4: SYNAPSIN 1 TRUNCATION IS ASSOCIATED WITH EPILEPSY, DEVELOPMENTAL DYSLEXIA AND LANGUAGE DISORDER

Reference :

Cossette P, Patry L, McDearmid JR, Ansaldo AI, Rouleau I, Sénéchal G, Marcotte K, Mottron L, Lapointe L, St-Onge J, Verlaan DJ, Gros-Louis F, Lortie A, Carmant L, Raskind WH, Nguyen DK, Drapeau P, and Rouleau GA. Synapsin 1 truncation is associated with developmental dyslexia, language impairment and epilepsy. Submitted.

Preface

"The problem of neurology is to understand man himself."
Wilder Penfield (1891-1976)

Garcia *et al* previously described a family with a nonsense mutation in *SYN1*, which was associated with epilepsy, aggressive behaviour, and learning disorder (Garcia et al. 2004). Unfortunatly, neither the epileptic syndrome, nor the specific impairments in cognitive function were described in details in this report. In this chapter, the second Human mutation in *SYN1* is described, in a large kindred segregating familial temporal lobe epilepsy with recessive X-linked inheritance. In these affected individuals, we found a Q555X mutation in synapsin 1 (*SYN1*), encoding for a neuron-specific phoshoprotein that associate with synaptic vesicles. By knocking-down *Syn1* in zebrafish, we showed that the $SYN1_{Q555X}$ mutation is associated with impaired synaptic transmission *in vivo*. Further characterization of mutation carriers revealed that males carrying $SYN1_{Q555X}$ mutations also exhibit developmental language disorder, whereas females exhibit dyslexia as a dominant X-linked trait. Overall, these data suggest that, in addition to epilepsy, mutations in *SYN1* may predispose towards a rare form of language disorder, including dyslexia in females. This finding may be an important step towards the unravelling of the molecular mechanisms underlying both oral and written language, the most distinctive features separating Humans from all other species.

Abstract

Synapsin 1 (*SYN1*) encodes for a neuron-specific phosphoprotein which interacts with synaptic vesicles. We report here a Q555X mutation in *SYN1* in a large French-Canadian kindred, AB, segregating language disorders and epilepsy. Affected females exhibit reading-spelling impairments compatible with developmental dyslexia, whereas affected males showed more severe language impairments, including deficits in reading-spelling, oral comprehension and expression, and pragmatics. Compared to wild-type synapsin, mutant *SYN1* showed abnormal synaptic transmission in an *in vivo* assay. These results suggest that truncated *SYN1* predisposes towards dominant X-linked dyslexia in females, and recessive X-linked language impairment and epilepsy in males.

Introduction

Dyslexia is the most common learning disorder, affecting approximately 5% of the general population (Shaywitz 1998). This language disorder is characterized by impaired reading skills in individuals showing otherwise normal intelligence, motivation, and access to education. Proficient reading requires the integration of both lexical (orthographic) and sub-lexical (phonological) processing routes. Whereas lexical processing allows global and fast decoding of written material, sub-lexical processing route involves phonological decoding, which is the ability to recognize the most elementary units of spoken language, called phonemes. Phonological processing is a core aspect of oral and written language development, since it allows discrimination, manipulation and understanding of speech sounds (Bishop and Adams 1990). Dyslexic

individuals are characterized by a deficit in one or both of these processing routes, but impaired phonological processing is the most consistent observation among poor readers (Habib 2000; Ramus 2001). In addition, dyslexia is frequently associated with spelling difficulties (dysorthographia), particularly for irregular words (e.g. yacht, brooch) and homophones (e.g see/sea, tail/tale) (Shaywitz 1998).

Despite extensive multidisciplinary studies, the specific mechanisms underlying developmental dyslexia remain a matter of debate (Habib 2000; Ramus 2001). An important genetic contribution has been consistently observed for dyslexia, as indicated by studies of familial aggregation and twins (DeFries et al. 1987; Shaywitz 1998). So far, up to nine putative loci have been mapped for dyslexia (Fisher and DeFries 2002). Recently, coding and non-coding variants thought to predispose to dyslexia have been reported in four genes, respectively mapping to the DYX1 (*DYX1C1*), DYX2 (*DCDC2*, *KIAA0319*), and DYX5 (*ROBO1*) loci (Taipale et al. 2003; Cope et al. 2005; Hannula-Jouppi et al. 2005; Schumacher et al. 2006). However, the relationship between these variants and the biological mechanisms underlying the disease is difficult to establish. The molecular mechanisms underlying dyslexia and language disorders thus remain to be determined.

Methods

Neuropsychological profiles, and assessment of oral and written language abilities in the AB family were performed by a team of neuropsychologists and speech-language pathologists blinded to the genetic status (Supplementary Table 1). The assessment of pragmatic difficulties was accomplished with the Pragmatic Profile of the Clinical

Evaluation Language Fundamentals (CELF-IV). Standardized Assessment for diagnosis of Autism (ADI-R & AGOS-G module 3) were performed in individuals IV-01 and IV-02. Clinical evaluations for epilepsy phenotype were routine. Collection of additional dyslexic families has been described elsewhere (Raskind et al. 2005).

Genotyping of microsatellite markers, linkage analysis, and gene sequencing were performed as previously described (Cossette et al. 2002). For temporal lobe epilepsy phenotype, we calculated two-points LOD scores with MLINK from the FASTLINK 3.0P package by using a penetrance of 0.8, a disease allele frequency of 0.00001, a phenocopy frequency of 0.0001 and equal allele frequencies. For language impairment phenotype, two-points LOD scores were calculated with a penetrance of 0.9 in males, 0.6 in females, a disease allele frequency of 0.0001, a phenocopy frequency of 0.001.

Cloning of *SYN1* cDNA and mutagenesis were performed as previously described (Cossette et al. 2002). Integrity of the constructs was tested by immublot analysis in COS7 cells transfected with wild-type and mutant *SYN1a* clones. The blot was probed with an anti-Synapsin1 antibody (SYSY, Synaptic Systems), as previously described (Cossette et al. 2002).

Syntenic *SYN1* in zebrafish was suppressed by micro-injection of an antisense morpholino in order to knock-down the function of the gene, as described elsewhere (Drapeau et al. 2002). To investigate underlying disruptions of the synaptic drive for locomotion, whole cell patch clamp methods were used to record spinal motoneurons following touch evoked swimming (Drapeau et al. 2002).

Results

Linkage analysis and molecular genetic studies

We identified a large family segregating temporal lobe epilepsy (TLE) over 4 generations, which was named AB, the initials of the proband (Supplementary Table 3). The mode of inheritance for TLE in this family is compatible with a recessive X-linked transmission (Fig 1A). We scanned the X chromosome by using 10 microsatellite markers, and found evidence for linkage on chromosome Xp11-q21. Fine mapping using additional markers identified key recombinants that allowed us to refine a candidate region of 58 Mb between markers *DXS8042* and *DXS6799*, with a maximum LOD score of 3.1 for marker *DXS6949*. By sequencing candidate genes mapping to the candidate interval we identified a stop codon (Q555X) in *SYN1*, encoding synapsin 1, in all TLE individuals from the family (Fig. 1C). This mutation was not detected in 400 control chromosomes.

Unexpectedly, detailed neuropsychological testing revealed severe impairment of reading and spelling in four TLE males carrying the $SYN1_{Q555X}$ mutation. This latter observation prompted us to systematically reassess the AB family with respect to language abilities and neuropsychological profiles. A total of 17 mutation carriers were evaluated, including six males and 11 females (Table 1 and supplementary Table 2). The IQ scores were within normal range of intelligence in all females and most of the males. In comparison to age-peers, the vast majority of mutation carriers (n=12 of 17) had poor reading performance with irregular, infrequent and polysyllabic words (sublexical processing deficits), together with impaired reading comprehension. In these poor readers, we also observed various degrees of lexical access deficits (e.g. poor naming and

fluency abilities), as well as striking dysorthographia. Overall, seven females (64%) carrying the *SYN1*$_{Q555X}$ mutation exhibit impaired performance in reading/spelling tasks, compatible with a diagnosis of dyslexia. In turn, five affected males (83%) showed deficits across many aspects of language, including poor performance in reading/spelling tasks, impaired oral comprehension, as well as abnormalities in the structure of sentences and discourse. In addition, all affected males (n=6) exhibit poor pragmatic language skills (social use of language). This combination of language deficits observed in males carrying the *SYN1*$_{Q555X}$ is qualitatively similar to those observed in autism-spectrum disorder (Kjelgaard and Tager-Flusberg 2001), and the diagnosis of pervasive developmental disorder was indeed confirmed in two affected males (33%). Speech dyspraxia was also present in 11 of the *SYN1*$_{Q555X}$ carriers.

Analysis of the phenotypes associated with the *SYN1*$_{Q555X}$ mutation in the AB family suggests more severe language impairments in males (Table 1 and supplementary Table 2), which is consistent with the fact that they carry a single mutated allele (hemizygous). In contrast, the heterozygous females show less severe language impairment, including 4 females that are asymptomatic. These observations in the AB family suggests that dyslexia is inherited as a dominant X-linked trait with reduced penetrance, while the same combination of language impairments and epilepsy is inherited as a recessive X-linked trait. Based on this broader definition of the phenotype, we found a LOD score of 3.84 for the *SYN1*$_{Q555X}$ mutation (Fig. 1B). We have also screened 96 well characterized males with familial dyslexia for mutation in *SYN1*, but no additional mutation was found.

Functional analysis of the SYN1$_{Q555X}$ mutation

Synapsins are a family of neuron-specific proteins that associate with both synaptic vesicles and the actin cystoskeleton. These proteins are mainly involved in the regulation of sustained neurotransmitter release (Gitler et al. 2004). Synapsins have also been implicated in neuronal development, synaptogenesis, and maintenance of mature synapses (Ferreira and Rapoport 2002). Two phosphorylation sites for Ca+/calmodulin-dependent protein kinase (CaMK) II reside in domain D of the protein (Fig 1C). Inactivation of these CaMKII sites has been shown to impair the binding of *SYN1* with both actin and synaptic vesicles (Chi et al. 2001). In addition, COOH-terminal regions of *SYN1* bind to Src homology 3 domains (SH3) (Onofri et al. 2000), and Rab3 proteins (Giovedi et al. 2004), which are believed to play an important role in the activity-dependent regulation synaptic vesicle recycling. Truncation of *SYN1* caused by the Q555X mutation is thus predicted to lead to dramatic changes in the binding properties of *SYN1*, which could impair neurotransmission.

We investigated whether the Q555X mutation alters the functional properties of *SYN1* by using zebrafish (*Danio rerio*), a well characterized model for the study of neurophysiological development (Drapeau et al. 2002). In contrast to *SYN1* knock-out in mice, in which mild changes in neurotransmitter release have been reported (Li et al. 1995), we found that knocking-down *Syn1* with a targeted antisense morpholino oligonucleotide (AMO) in *Danio rerio* caused a dramatic behavioural phenotype. In 71% (n = 32 of 47) of *Syn1* AMO injected fish, disruption of touch-induced swimming behaviour was observed (Fig. 2C). Recordings of spinal motoneurons revealed that *SYN1* AMO injected fish received a transient sensory-evoked response but this failed to generate a

sustained, rhythmic synaptic drive seen in wild type motoneurons following touch (n= 8 of 8, Fig. 2D). In contrast, fish that were injected with a control mismatch AMO (n = 27 of 27, not shown) had wild type patterns of swimming behaviour and synaptic drive. Coinjection of wild-type human *SYN1a* mRNA with the *SYN1* AMO significantly reduced the number of fish that displayed a strong disruption of swimming behaviour to 44% (n = 28 of 63, Fig. 2E) and whole cell recording revealed a restoration of sustained synaptically driven activity in these fish (n = 5 of 5, Fig. 2F). In contrast, only 20% (n = 14 of 71) of fish coinjected with human $SYN1_{Q555X}$ RNA and *SYN1* AMO generated swimming in response to touch (Fig. 2G). The majority of recorded motoneurons of these fish (n = 5 of 6) showed a transient sensory-mediated response but this failed to generate sustained synaptic activity (Fig. 2H). These results are compatible with a loss of function of the $SYN1_{Q555X}$ mutation and impairment of synaptic transmission within the central nervous system.

Discussion

Garcia *et al* previously reported a W356X mutation in *SYN1*. As for the AB family described here, males carrying the $SYN1_{W356X}$ mutation not only have epilepsy, but also learning difficulties (Garcia et al. 2004). We believe that there is now compelling evidences supporting that truncated *SYN1* is responsible for both epilepsy and learning disorders. Indeed, truncated mutations in *SYN1* have been independently found in two large kindred, the phenotype associated with these two mutations is similar, and we show here that $SYN1_{Q555X}$ mutation leads to a loss of function.

Unfortunately, Garcia *et al* did not report the learning abilities of females, nor did they provide detailed neuropsychological profiles in mutation carriers (Garcia et al. 2004).

Here, we show that almost all affected individuals of the AB family exhibit striking deficits in core aspects of language, such as lexical (orthographic) and sub-lexical (phonological) processing routes. More importantly, we have found similar deficits, although less severe, in the majority of females carrying the *SYN1*$_{Q555X}$ mutation. Overall, the language 'symptomatology' is qualitatively similar in all affected individuals with more severe impairments in males, an observation that is biologically plausible for a X-linked disorder. The absence of non-verbal cognitive impairment or mental retardation in affected individuals, and the fact that language impairments were observed in 8 non-epileptic individuals from the AB family, highlights the language-specific nature of the *SYN1*$_{Q555X}$ mutation induced phenotype.

The clinical manifestations in affected individuals for the AB family clearly extend beyond the established diagnostic criteria for a single developmental language disorder. However, because the biology of these disorders remains largely unknown, these criteria remain empirical and are still a matter of debate. By rigorously using these diagnostic categories, *SYN1*$_{Q555X}$ mutation would be associated with *a priori* different neurodevelopmental disorders, including dyslexia (7 females), specific language impairments (3 males), and pervasive developmental disorder (2 males) (Table 1). Based on familial aggregation studies (Piven et al. 1997; Folstein et al. 1999) and language profiles observed in autistic individuals (Kjelgaard and Tager-Flusberg 2001), several authors suggested these disorders may potentially share genetic mechanisms. Although additional genetic and environmental factors may be involved in the AB family, the clinical phenotype observed is consistent with this latter hypothesis. Moreover, the

phenotype observed in $SYN1_{Q555X}$ mutation carriers is not so heterogeneous if we analyse the genders separately (Table 1).

Notwithstanding the classification considerations, the data presented here support that *SYN1* is a novel gene associated with language disorder. The fact that we did not find mutations in additional families does not invalidate this finding. This most probably reflects the fact that a majority of dyslexic families exhibit a complex inheritance pattern (Fisher and DeFries 2002), whereas *SYN1* may predispose towards a rare form of dyslexia with Mendelian inheritance.

Mice lacking *SYN1* show increased seizure propensity, learning deficits and abnormal synaptogenesis (Li et al. 1995; Gitler et al. 2004). Among synapsins, *SYN1* is the most abundant and is highly expressed in the limbic system and neocortex. We speculate that abnormal development of the temporal lobe may be the common biological mechanism underlying epilepsy and learning disorder. The proper development of the speech areas may be particularly sensitive to disruption of synaptic transmission. If reduced activity-dependent development underlies language impairments found in family AB, this might account for the lack of dominant hemispheric growth of the dyslexic brain. Indeed, individuals with either dyslexia or autism lack the normal asymmetry of the planum temporale (Habib 2000; Rojas et al. 2005), a key component of the receptive language within the left hemisphere.

In conclusion, we have identified a mutation leading to truncation of the pre-synaptic protein *SYN1* in a large family segregating dyslexia, language impairment and epilepsy. Mutations in two isoforms of neuroligin (*NLG3*, *NLG4*), encoding for post-synaptic cell-adhesion proteins have been recently found in families with autism (Jamain et al. 2003).

Impaired synaptic transmission may thus be a common mechanism underlying language disorders and autism. Although these findings so far implicate a small number of families, the identification of such molecular mechanisms have implications in the search for additional genes predisposing to these neurodevelopmental disorders.

Acknowledgements

The authors wish to thank the families for their participation in this study. We are also grateful to Micheline Gravel for her implication in the collection of blood samples, and clinical information. We also thank Caroline Meloche and Josée Poirier for technical assistance. P.C., G.A.R., and P.D. are supported by Canadian Institute for Health Research (CIHR). L.P. is supported by the Savoy Foundation.

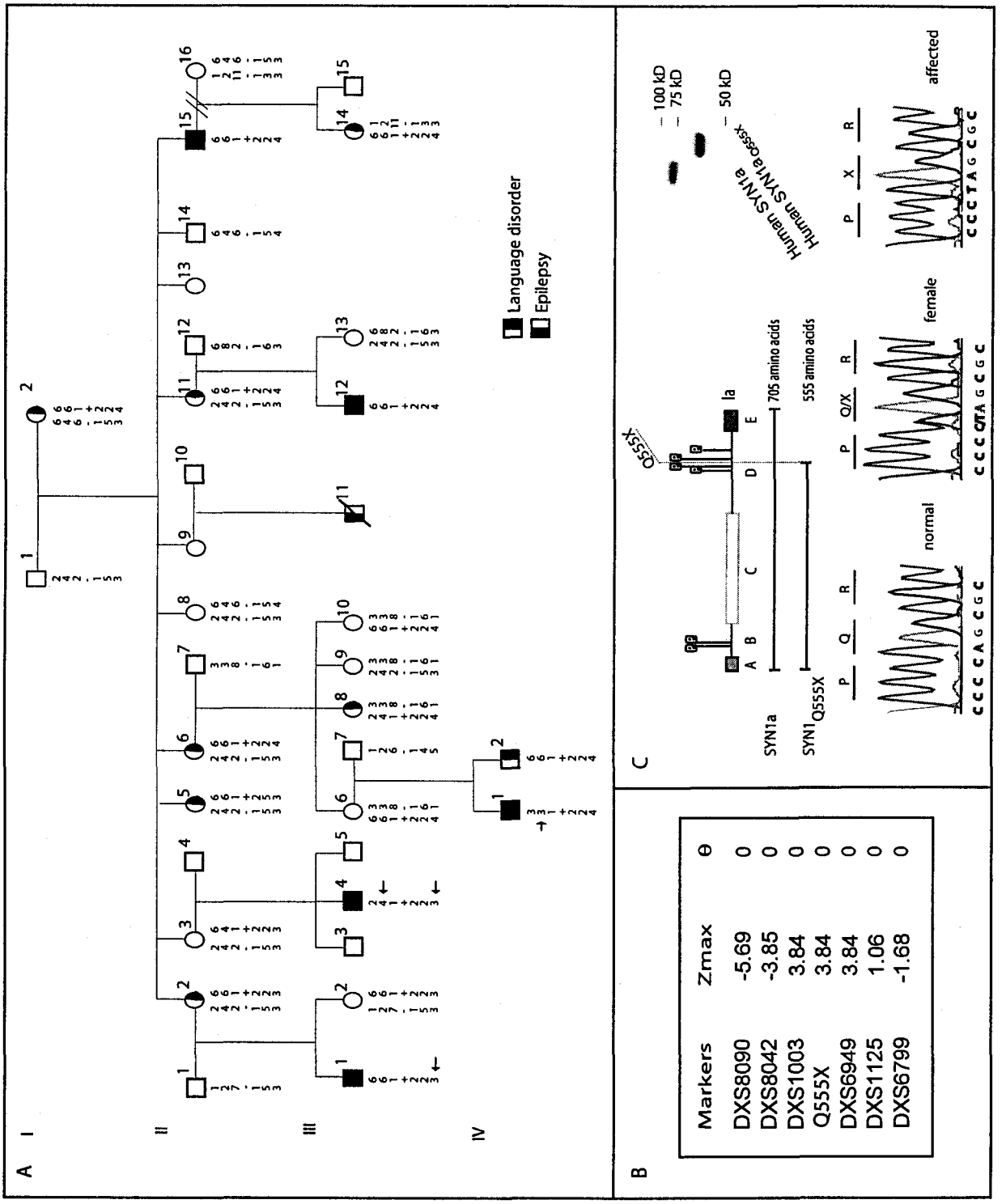

A
I
II
III
IV
Language disorder
Epilepsy
B
Markers Zmax θ
DXS8090 -5.69 0
DXS8042 -3.85 0
DXS1003 3.84 0
Q555X 3.84 0
DXS6949 3.84 0
DXS1125 1.06 0
DXS6799 -1.68 0
C
100 kD
75 kD
50 kD
Human SYN1a
Human SYN1aQ555X
Q555X
Ia
A B C D E
705 amino acids
555 amino acids
SYN1a
SYN1Q555X
P Q R
P Q/X R
P X R
normal
female
affected

Figure 1. Identification of a Q555X mutation *SYN1* in a large family with X-linked language impairment and epilepsy. In panel A, consistent haplotypes segregating with dyslexia in females, as well as language impairment and epilepsy in affected males. Alleles were numbered according to their respective size corresponding to the CEPH genotyping database. Key recombinants are indicated by arrows. In panel B, the maximum lod scores obtained with these markers and for the Q555X mutation (+). In panel C, sequence analysis from a normal control, female carrier, and affected male. The female and male carriers are respectively heterozygous and hemizygous for a C → T substitution, which results in a stop codon (TAG) in amino acid 555 of the predicted protein (Q555X). Immunoblot analysis shows that the Q555X mutation leads to truncated *SYN1*. Functional domains and phosphorylation sites of *SYN1* are shown.

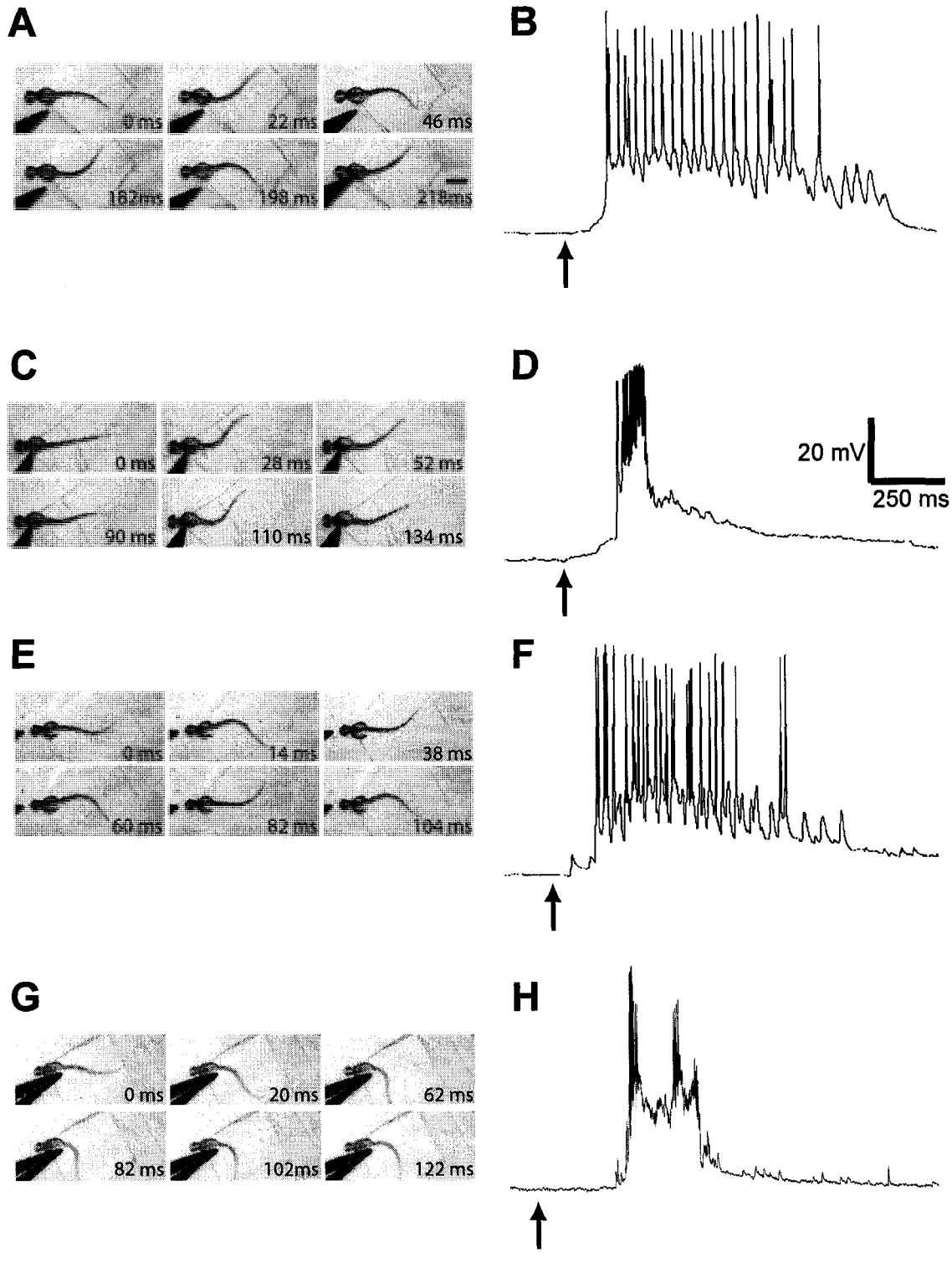

A
0 ms
22 ms
46 ms
162ms
198 ms
218ms
B
C
0 ms
28 ms
52 ms
90 ms
110 ms
134 ms
D
20 mV
250 ms
E
0 ms
14 ms
38 ms
60 ms
82 ms
104 ms
F
G
0 ms
20 ms
62 ms
82 ms
102ms
122 ms
H

Figure 2. Disruption of *SYN1* induces motor phenotypes in *Danio rerio*. A. Selected frames showing rhythmically alternating flexions of the trunk during touch-evoked swimming in wild type zebrafish at 2 days in development. Swimming was elicited by touching the trunk with a pair of fine forceps (seen in the bottom left corner). B. Rhythmic synaptic drive in a motoneuron of a 2 day wild type fish following touch (arrow). C. Touch does not elicit rhythmic trunk flexions in *Syn1* AMO injected fish at 2 days. D. Absence of touch-evoked (arrowed) sustained, rhythmic synaptic drive in *Syn1* AMO injected fish at 2 days. E. Sustained, rhythmic swimming activity in a 2 day fish coinjected wild type *SYN1* mRNA and *Syn1* AMO. F. Sustained, rhythmic synaptic activity in motoneuron of a 2 day fish coinjected with *Syn1* AMO and *SYN1* mRNA. G. Touch does not elicit rhythmic trunk flexions in 2 day fish coinjected with $SYN1_{Q555X}$ mRNA and *Syn1* AMO. H. Absence of sustained, rhythmic synaptic drive in a motoneuron following touch (arrowed) in 2 day fish coinjected with $SYN1_{Q555X}$ mRNA and *Syn1* AMO. Scale bar in A,C,E,G = 250 μm.

ID	Gender	Reading-spelling impairments	Oral comprehension and expression impairments	Pragmatics difficulties	Specific developmental disorder
I-02	F	yes	no	no	dyslexia¤
II-02	F	yes	no	no	dyslexia
II-03	F	no	no	no	-
II-05	F	yes	no	no	dyslexia
II-06	F	yes	no	yes	dyslexia
II-11	F	yes	no	no	dyslexia
III-02	F	no	no	no	-
III-06	F	no	no	no	-
III-08	F	yes	no	no	dyslexia
III-10	F	no	no	no	-
III-14	F	yes	yes	no	dyslexia†
II-15	M	yes	yes	yes	SLI
III-01	M	yes	yes	yes	SLI
III-04	M	no	no	yes	-
III-12	M	yes	yes	yes	SLI
IV-01	M	yes	yes	yes	PDD*
IV-02	M	yes	yes	yes	PDD*

Table 1. Evaluation of language abilities in family AB.

All individuals carrying the Q555X mutation in *SYN1* underwent neuropsychological evaluation and detailed assessment of oral and written language. Compared to age-peers, the vast majority of mutation carriers (n=12) had impaired performance in reading/spelling tasks, despite normal intelligence. A total of five females carrying the $SYN1_{Q555X}$ mutation meet criteria for developmental dyslexia. In two additional females, impaired language abilities and reading performance were also compatible with dyslexia, but either young age (†) or inadequate educational opportunity (¤) preclude a definite diagnosis. Language impairment was more severe in five males (individuals II-15, III-01, III-12, IV-01 and IV-02), who showed receptive and expressive language deficits compatible with a diagnosis of specific language impairment (SLI). The pattern of language deficits was qualitatively similar in individuals IV-01 and IV-02 (*). In principle, SLI cannot be diagnosed in children with pervasive developmental disorder (PDD). However, previous analysis of language impairment in individuals with autism (Kjelgaard and Tager-Flusberg 2001), as well as familial aggregation studies (Piven et al. 1997; Folstein et al. 1999) suggest that dyslexia, SLI, and autism-spectrum disorder may share common genetic mechanisms. The clinical phenotypes of the AB family presented here are consistent with this hypothesis.

Domain	Test	Tasks
Neuropsychological assessment	Wechsler Adult Intelligence Scale®-Third Edition (WAIS®–III)	Picture completion, Block design, Similarities, Arithmetic, Information, Substitution, Digit span
	Wechsler Intelligence Scale for Children®-Fourth Edition (WISC®-IV)	Block design, Similarities, Digit Span, Picture concepts, Coding, Matrix reasoning, Letter-Number sequencing, Vocabulary, Comprehension, Symbol search
	Wecshler Memory Scale®-Third Edition (WMS®-III)	Mental control (Alphabet, Numbers, Days of the week)
	Rey Auditory Verbal Learning Test (RAVLT)	15 words of Rey
	Letter A cancellation test	Visual selective attention
	Outil de dépistage des dyslexies-ODEDYS (test of screening dyslexia)	Letter sequences discrimination, Dictation of regular and irregular words and pseudowords.
	NEuroPSYchological investigation for children®-NEPSY®	Phonological awareness (phonological processing abilities)
	Color-Word interference test from the Delis-Kaplan Executive Function System™ (D-KEFS™)	Color naming and word reading
	Culver form (for adults)	Right-Left discrimination
	Épreuve Verbale d'Aptitudes Cognitives-EVAC (Verbal test of cognitive aptitudes)	Right-Left discrimination
	Verbal fluency test	Letter fluency (P) and category fluency (animals)
Oral language	Test of word finding for adolescents and adults-TWF	Receptive and expressive vocabulary
	Boston Naming Test (30)	Oral denomination and written denomination
	Clinical Evaluation Language Fundamentals-IV-CELF-4	Sentence comprehension, Expressive vocabulary, Sentence production
Written language	LOBROT L3 (norms from BELEC)	Written sentence comprehension
	Bébé d'Edmonton history	Written discourse comprehension
Reading	Batterie d'évaluation du langage écrit et de ses troubles-BELEC: MIM A (Belgian battery of evaluation of the written language and its disorders)	Word and pseudoword reading, according to frequency, length and graphems complexity
	Batterie d'évaluation du langage écrit et de ses troubles-BELEC: REGUL (Belgian battery of evaluation of the written language and its disorders)	Regular and irregular word reading
	Chapman-Cook Speed of Reading Test	Written comprehension and reading speed
	L'Alouette	Reading fluency
Writing	Batterie d'évaluation du langage écrit et de ses troubles-BELEC: ORTHO3 (Belgian battery of evaluation of the written language and its disorders)	Writing words
	Test de Rendement pour Francophones-TRF (french version of the Wide-Range Achievement Test-WRAT)	Orthography
	No specific protocol	Words and Sentences dictation
Pragmatic language	Clinical Evaluation Language Fundamentals-IV-CELF-4	Pragmatics profile questionnaire

Supplementary Table 1. Neuropsychological and language abilities assessment. Neuropsychological and language assessment in the AB family were performed by neuropsychologists and speech-language pathologists blinded to the genetic status. Neurospychological assessment included the WAIS III and WISC IV. Assessment of oral and written language abilities was completed with a battery of tasks and tests widely used with the francophone population. More precisely, assessment focused on: a) lexical access abilities, as revealed by confrontation naming and fluency tasks (phonemic and semantic fluency compared to age peers), b) oral expression abilities at the sentence and discourse levels (sentence and discourse structure in conversational and narrative discourse) c) comprehension abilities at the sentence and discourse levels (adaptation of CELF IV for children and Phrase structure in French) d) reading –spelling abilities, as revealed by pseudo-words, polysyllabic and irregular words (BELEC), and d) spelling regular, irregular and pseudo-words words (BELEC). Pragmatic abilities were examined with the Pragmatic Profile of the Clinical Evaluation Language Fundamentals (CELF-IV).

					READING TESTS				SPELLING TESTS			
ID	Gender	Age	Years of education	IQ/VIQ/PIQ	Pseudo-words	Polysyllabic words	Complex graphemes	Compre-hension	Contextual and constant spelling	Unfrequent irregular orthography	Morphologic ally based orthography	Non-morphologic ally based orthography
II-02	F	44	14	97/97/95	**	**	**	**	**	**	**	**
II-03	F	56	12	96/99/93	**	**	**	NA	NA	NA	NA	NA
II-05	F	53	8	87/77/100	**	**	**	**	**	*	**	**
II-06	F	54	12	89/95/83	**	**	**	**	**	0	**	0
II-11	F	46	12	91/91/92	**	**	**	*	**	*	**	**
III-02	F	14	8	105/102/109	**	**	**	**	**	**	**	**
III-06	F	31	12	95/93/97	0	0	0	0	NA	NA	NA	NA
III-08	F	22	15	100/98/104	**	**	**	**	NA	NA	NA	NA
III-10	F	28	13	98/91/109	**	**	**	NA	NA	NA	NA	NA
III-14	F	7	1	91/73/111	**	**	**	NA	**	**	**	**
II-15	M	41	9	78/79/80	**	**	**	**	**	**	**	**
III-01	M	20	8	78/83/76	**	**	**	**	**	**	**	**
III-04	M	30	15	104/122/84	0	**	**	NA	NA	NA	NA	NA
III-12	M	22	9	78/82/78	**	**	**	**	NA	NA	NA	NA
IV-01	M	8	1	51/45/72	NA	NA	NA	NA	NA	NA	NA	NA
IV-02	M	6	Preschool	65/71/70	NA	NA	NA	NA	NA	NA	NA	NA

Supplementary Table 2. Summary of neuropsychological and language ability profiles found in individuals carrying the *SYN1*$_{Q555X}$ mutation. Scores of reading and spelling tests were obtained from the BELEC battery, except for reading comprehension, which were obtained from Lobrot test. Results from reading and spelling tests are presented using z point comparison to mean control group: '0' $z \geq -1.0$; * $-2.0 \leq z < -1.0$; ** $z < -2.0$. For children, we used control values available from the BELEC battery. For adult, comparison was made by using our own control group of 20 healthy individuals from the Quebec population, matched for age and years of education. Abreviations used: F, female; M, male; NA, not available

ID	Gender	Age	Age of onset	Febrile seizures	Spontaneous seizures	Reflex seizures	Trigger	ii-EEG	i-EEG	MRI	Ictal SPECT
II-02	F	44	-	no	no	no	-	-	-	-	-
II-03	F	56	3	yes	no	no	-	NA	NA	normal	NA
II-05	F	53	-	no	no	no	-	-	-	-	-
II-06	F	54	-	no	no	no	-	-	-	-	-
II-11	F	46	-	no	no	no	-	-	-	-	-
III-02	F	14	-	no	no	no	-	-	-	-	-
III-06	F	31	-	no	no	no	-	-	-	-	-
III-08	F	22	NA	yes	no	no	-	NA	NA	normal	NA
III-10	F	28	-	no	no	no	-	-	-	-	-
III-14	F	7	-	no	no	no	-	-	-	-	-
II-15	M	41	1	yes	CPS	CPS, GTCS	shower, wet towel	normal	right temporal	right HA	right temporal
III-01	M	20	4	no	CPS	CPS, GTCS	bath, shower	normal	NA	normal	NA
III-04	M	30	13	no	CPS, GTCS	no	-	normal	diffuse	normal	NA
III-12	M	22	14	no	CPS	CPS, GTCS	bath, shower	normal	NA	NA	NA
IV-01	M	8	4	no	CPS	CPS	bath, shower	normal	diffuse	left HA	left temporal
IV-02	M	6	-	no	no	no	-	-	-	-	-

Supplementary Table 3. Summary of epilepsy phenotype found in individuals carrying the *SYN1*$_{Q555X}$ mutation. Complex partial seizures (CPS) in affected individuals were characterized by chilling aura, alteration of consciousness with either fixed gaze or ocular revulsion, oral and manual automatisms, hypotonia, and cyanosis. These complex partial seizures may infrequently be prolonged by secondary generalized tonic-clonic seizures (GTCS) in affected individuals. Other abbreviations: iiEEG, interictal electroencephalogram; iEEG ictal electroencephalogram; MRI, magnetic resonance imaging of the brain; SPECT, single-photon emission computerized tomography of the brain; HA, hippocampal atrophy; F, female; M, male; NA, not available.

CHAPTER 5: CONCLUSION

Although epilepsy overall is considered to be a complex genetic trait, there is increasing evidences supporting the existence of monogenic forms of epilepsy in Humans (Gourfinkel-An et al. 2004; Turnbull et al. 2005). Previous genetic studies using conventional positional cloning approaches allowed the identification of genes predisposing to a variety of familial epilepsies, including benign neonatal familial convulsion syndrome (BNFC) (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), (Steinlein et al. 1995; De Fusco et al. 2000), generalized epilepsy with febrile seizures (GEFS) (Wallace et al. 1998; Escayg et al. 2000b), and autosomal dominant partial epilepsy with auditory features (ADPEAF) (Kalachikov et al. 2002). In this thesis, additional data supporting the existence of monogenic epilepsies is provided, with the identification of novel mutations in *SCN1A*, *GABRA1*, and *SYN1*. Mutations in these genes are predicted to lead to epilepsy through a variety of molecular mechanisms. Most of the work presented here was performed between January 2001 and June 2005. Over this short period of time, at least 12 novel genes have been associated with idiopathic epilepsy (Table 7) (Heron et al. 2002; Haug et al. 2003; Dibbens et al. 2004; Suzuki et al. 2004). All of these successful examples are in contrast with the poor harvest provided by association studies using large cohorts of epileptic individuals (Mulley et al. 2005). Indeed, although there have been several reports of genes associated with various idiopathic epilepsies (Pal et al. 2003; Greenberg et al. 2005), these studies await replication. More importantly, the biological

mechanisms by which these genetic variations lead to the disease remain to be determined.

Genetic heterogeneity in monogenic and complex epilepsies

One potential limitation of mapping and cloning genes underlying monogenic epilepsies is that it may allow identification of genes predisposing only for rare forms of the disease. Indeed, mutations in epilepsy genes identified so far explain only a minority of all familial epilepsies. As an example, following the identification of the A322D mutation in *GABRA1* described in chapter 3, we screened 150 individuals with IGE for mutations in *GABRA1*, including 21 familial cases. Only one other mutation was found, in a small IGE family (Cossette *et al*, in preparation). By screening a cohort of 98 individuals with IGE (including 60 families), Maljevic *et al* described another mutation in *GABRA1* in a sporadic case with childhood absence epilepsy (Maljevic et al. 2006) (appendix 2). Based on these data, mutations in *GABRA1* would be responsible for approximately 2% of familial IGE, and less than 1% of all IGE cases overall. However, other groups of investigators failed to identify mutations in *GABRA1*, suggesting that frequency of mutation in this gene would be even lower (Marini et al. 2004; Ito et al. 2005; Ma et al. 2006).

Mutations in *GABRG2* have been described in at least three different families with childhood absence epilepsy (CAE), one of the classical IGE phenotype (Wallace et al. 2001a; Harkin et al. 2002; Kananura et al. 2002). In addition, recent studies have shown that mutations in *CLCN2* gene (voltage-gated chloride channel) can predispose towards a variety of classical IGE phenotypes, in five different families (Haug et al. 2003; D'Agostino et al. 2004). As for *GABRA1*, it appears that mutations in these genes

represent a rare cause of familial IGE (D'Agostino et al. 2004; Marini et al. 2004). One notable exception to this rule is the relatively high number of mutations found in *SCN1A*. Indeed, mutations in this gene have been found in a significant proportion of individuals with generalized epilepsy with febrile seizures plus (GEFS+; 10%) and severe myoclonic epilepsy of infancy (SMEI; 50%) (Turnbull et al. 2005). However, in contrast to IGE, these two latter syndromes are very rare.

Overall, even if the conventional positional cloning approach allowed the identification of an increasing number of genes predisposing to idiopathic epilepsy, including for the common IGE phenotypes, systematic screening of these genes in larger cohort has yielded very few mutations. Clearly, this method did not lead to the identification of major gene(s) responsible for common IGE cases. We propose two potential explanations, not mutually exclusive, for this observation:

(1) familial epilepsy is probably an extremely *heterogeneous genetic disorder*. So far, at least 20 genes have been identified for monogenic epilepsy, explaining only a minority of all familial cases (Gourfinkel-An et al. 2004; Turnbull et al. 2005). This is not surprising, considering that epilepsy results from a dysfunction of the cerebral cortex, a very complex anatomical structure. In addition, previous neurophysiological studies of epileptic brains revealed that a variety of different mechanisms can lead to impaired neuronal excitability (see p. 19). Moreover, there is mounting evidences that other neurogenetic disorders also exhibit such a high genetic heterogeneity. As an example, autosomal dominant spinocerebellar ataxias and muscular dystrophy have been associated with 29 and 27 loci respectively (Emery 2002; Duenas et al. 2006), although these disorders are caused by a dysfunction of much less complex structures of the

nervous system (e.g. cerebellum, muscle). Based on these observations, it is reasonable to hypothesize that a *high number of different genes* will be responsible for the disease;

(2) the majority of genetic epidemiology studies suggest that common forms of epilepsy are caused by genetic variation in several genes together with environmental factors, features of a *complex trait* (see p. 34). Should this be the case, conventional positional cloning methods will invariably miss major genes relevant for epilepsy, because it does not allow the detection of *genetic variants associated with smaller effect on the phenotype*, as expected in polygenic epilepsies. These studies on monogenic epilepsies have nonetheless led to the identification of specific molecular mechanisms responsible for the disease. In addition, genotype-phenotype correlations provided food for revision of clinical classification of epileptic syndromes.

Clinical heterogeneity and classification of epileptic syndromes

Detailed clinical evaluation of families with epilepsy has been found to be of great value in the identification of epilepsy genes. However, the current classification of epileptic syndromes has been challenged by the findings of molecular genetics. The most striking example is mutations in the *SCN1A* gene, which have been found to cause a large spectrum of epileptic syndromes, including: febrile seizure alone, idiopathic generalized epilepsy, partial epilepsy, myoclonic-astatic epilepsy, and severe myoclonic epilepsy of infancy (Escayg et al. 2000b; Claes et al. 2001; Sugawara et al. 2001a; Sugawara et al. 2001b; Wallace et al. 2001b). All of these epileptic syndromes were *a priori* very different in terms of clinical manifestations, intellectual disturbance, and prognosis. It is only *a posteriori* that febrile seizure has been found to be present in all individuals with

mutation in *SCN1A*. Therefore, although the study and grouping of specific epileptic syndromes have been initially found to be successful for the identification of epilepsy genes, further studies revealed that the same gene could predispose towards various epileptic syndromes that were believed to be distinct. Whereas mutations in EFHC gene that are associated with classical JME only, (Suzuki et al. 2004), mutations in most of the IGE genes identified so far (*GABRA1, GABRG2, GABRD*, and *CLCN2*) are rather associated with a variety of classical IGE phenotypes, including juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE), childhood absence epilepsy (CAE), and epilepsy with grand mal seizures on awakening (EGMA) (Table 10) (Baulac et al. 2001; Wallace et al. 2001a; Cossette et al. 2002; Haug et al. 2003; Dibbens et al. 2004; Maljevic et al. 2006). Several observational studies previously proposed that these syndromes, although considered to be distinct entities in the current classification, rather represent a biological continuum (see p.6). Genotype-phenotype correlations, although from a limited number of families, further support this hypothesis. However, additional data will be needed in order to explore potential overlap between these various epileptic syndromes. Eventually, these findings should contribute to a revised, molecular-based, classification of IGE.

Table 10. Monogenic forms of classical idiopathic generalized epilepsy

Genes	Phenotype	Reference
GABRA1	JME, CAE	Cossette, 2002; Maljevic, 2006
GABRG2	GEFS+, CAE	Baulac, 2001; Wallace, 2001a; Harkin, 2002; Kananura, 2002
GABRD	GEFS+, JME	Dibbens, 2004
CLCN2	JME, JAE, EGMA, CAE	Haug, 2003; D'Agostino, 2004
EFHC	JME	Suzuki, 2004

Endophenotypes: the case of learning disorders

Nonsense mutations in *SCN1A* have been systematically associated with severe myoclonic epilepsy of infancy (SMEI), a severe form of epilepsy associated with mental retardation (Claes et al. 2001; Turnbull et al. 2005). A small proportion of individuals with less severe mutation in the *SCN1A*, including the D188V described in chapter two, also exhibit mental retardation (Wallace et al. 2001b; Cossette et al. 2003). These observations raise the possibility that mutations in *SCN1A* not only predispose to epilepsy, but also to impaired cognition. However, based on animal studies, many authors argued that cognitive and behavioural impairments observed in epileptic individuals may result from repetitive seizures and/or the administration of anti-epileptic drug in the developing brain (see p. 16). Together with additional yet unidentified environmental factors, these may thus be responsible for impaired cognition in individuals with mutation in *SCN1A*.

Detailed examination of individuals carrying the Q555X mutation in *SYN1*, described in chapter 4, unexpectedly revealed a high frequency of a specific language disorder. Whereas learning disorders and mental retardation are common findings in epileptic population, specific association with dyslexia and specific language impairment has been rarely reported (see p.18). In turn, autism-spectrum disorder is more common in epileptic individuals, compared to the general population (Olsson et al. 1988; Rapin 1997). In chapter 4 of this thesis, we have shown that seven females and one male carrying the Q555X mutation in *SYN1* respectively exhibit dyslexia and autism-spectrum disorder, even though they never experienced seizures. This observation suggests that mutation in *SYN1* not only predisposes to temporal lobe epilepsy, but also to reading impairment, and potentially autism-spectrum disorder *in the absence of epilepsy*. Interestingly, *SYN1*

knock-out mice not only exhibit reduce seizure threshold, but also impaired learning process (Baldelli et al. 2005). Screening of additional cases with various learning disorders will be needed in order to validate this hypothesis in Humans.

Impaired membrane excitability caused by mutations in sodium channels

So far, 14 missense mutations in *SCN1A* have been identified, accounting for approximately 10% of GEFS+ families tested (Turnbull et al. 2005). In functional assays, these missense mutations produce subtle changes in biophysical parameters of channel activity such as increased persistent current and alterations in voltage-dependent gating (Spampanato et al. 2001; Lossin et al. 2002; Lossin et al. 2003; Spampanato et al. 2003). One GEFS+ mutation in the C-terminal cytoplasmic domain of *SCN1A* was shown to reduce interaction with the β1 subunit (Spampanato et al. 2004). Electrophysiological analysis of D188V mutant channels expressed in HEK cells, as described in chapter two of this thesis, revealed that this mutation causes decreased cumulative inactivation of sodium current during high frequency channel activity, an effect compatible with membrane hyperexcitability.

In addition to inherited mutations, it is now clear that *de novo* mutations of *SCN1A* account for approximately 50% of patients with severe myoclonic epilepsy of infancy (SMEI), a severe, early-onset epilepsy accompanied by intellectual deterioration (Claes et al. 2001; Sugawara et al. 2002). Nearly 200 independent mutations have been identified in affected children, and more than 90% of tested cases were sporadic (Turnbull et al. 2005). Approximately half of the SMEI mutations are nonsense mutations resulting in truncation of the channel protein and loss of channel activity. Phenotypic severity is

comparable for truncations close to the N-terminus of the protein and those close to the C-terminus (Turnbull et al. 2005). In addition, complete or partial deletion of SCN1A have been recently found in individuals with SMEI (Madia et al. 2006; Mulley et al. 2006), suggesting that haploinsufficiency and loss of function of *SCN1A* are responsible for SMEI. Furthermore, it was recently shown that many missense mutations in SMEI patients also result in a loss of function (Rhodes et al. 2004; Ohmori et al. 2006). Taken together, these data suggest that the spectrum of severity of *SCN1A*-related epilepsies could be partially explained by the severity of sodium channel dysfunction, culminating with the devastating loss-of-function mutations found in SMEI.

A few mutations have been identified in the closely related channel *SCN2A*, located 600 kb downstream from *SCN1A* (Turnbull et al. 2005). One missense mutation in *SCN2A* was found in a GEFS+ family (Sugawara et al. 2001b), and six missense mutations were identified in patients with benign familial neonatal-infantile seizures, a mild syndrome that presents and remits in the first year of life (Heron et al. 2002; Berkovic et al. 2004). Finally, one truncation mutation in *SCN2A* was identified in a patient with intractable epilepsy resembling SMEI (Heron et al. 2002; Berkovic et al. 2004).

The Na channel β subunits (β1-4) are small transmembrane proteins with an extracellular IgG loop. Association with the β subunit influences α subunit trafficking, stability, and channel gating (Isom 2001). Two different mutations in the β1 gene *SCN1B* have been identified in patients with GEFS+ (Wallace et al. 1998; Audenaert et al. 2003). As many of the *SCN1A* missense mutations found in GEFS+ patients, it seems that the major effect of these mutations is to delay Na channel inactivation.

Impaired synaptic transmission caused by mutations in $GABA_A$ receptor subunits

$GABA_A$ receptors are ligand-gated chloride channels that mediate fast inhibition in the central nervous system. Their molecular structure comprises a heteropentameric protein complex assembled from 17 different classes of subunits (α1-6, β1-4, γ1-3, δ, ε, π, and θ). So far, epilepsy-causing mutations have been identified in *GABRG2* (Baulac et al. 2001; Wallace et al. 2001a; Harkin et al. 2002; Kananura et al. 2002), *GABRA1* (Cossette et al. 2002; Maljevic et al. 2006) and *GABRD* (Dibbens et al. 2004) encoding respectively the α1, γ2 and δ subunits. As described for the $GABRA1_{A322D}$ mutation in chapter two of this thesis, *in vitro* functional studies have revealed that the majority of these mutations result in a reduction of GABA-activated chloride currents. In at least two mutations in *GABRG2* (Q351X, R43Q) (Harkin et al. 2002; Kang and Macdonald 2004) and two mutations in *GABRA1* (A322D, S326fs328X) (Krampfl et al. 2005; Maljevic et al. 2006), it has been shown that the reduction in the amplitude of GABA-evoked current was due to reduced surface expression of receptor protein, caused by retention of mutant receptors in the endoplasmic reticulum. Finally, in *GABRD*, two missense mutations are associated with GEFS+ (Dibbens et al. 2004). One of these *GABRD* mutations (E177A), like mutations in *GABRG2* and *GABRA1*, results in decreased amplitude of GABA-evoked currents, but the other (R220C) does not. Whether it is a rare neutral variant, or is associated with more subtle effects on the $GABA_A$ receptor remains to be determined. In the same study, another variant in *GABRD* (R220H) was detected in a JME family, but it was also present in the general population with a frequency of 4.2% (Dibbens et al. 2004). Interestingly, this R220H polymorphism is nonetheless associated with reduced peak amplitude of GABA-evoked current, which is expected to increase neuronal

excitability. The role of this functional polymorphism in epilepsy with complex inheritance needs to be clarified by larger cohort of IGE individuals. However, this and similar functional polymorphisms may represent candidate genes with small effect on the phenotype, as it is anticipated for common epilepsies with complex inheritance, such as JME and CAE.

Surprisingly, knock-out of genes encoding for various $GABA_A$ receptor subunits, including $\alpha 1$, $\alpha 5$, $\gamma 3$, or δ, are not associated with epileptic phenotype in mice (Culiat et al. 1994; Collinson et al. 2002; Kralic et al. 2002; Peng et al. 2002; Noebels 2003). However, targeted deletion of *GABRG2* in mice is lethal after birth, and mutants exhibit abnormal episodes of hyperactivity that might be compatible with neonatal seizures (Gunther et al. 1995; Oberbauer et al. 2003). In addition, deletion of $\beta 3$ in mice is associated with severe seizure disorder (Homanics et al. 1997; DeLorey et al. 1998). So far, no mutation has been described in *GABRB3* in Humans.

Impaired membrane excitability caused by mutations in chloride channels

Five epilepsy mutations have been identified in the *CLCN2* gene, three of which have been subject to functional studies (Haug et al. 2003; D'Agostino et al. 2004). *CLCN2* is encoding the voltage-gated chloride channel ClC-2, which is strongly expressed in the brain, and notably localized in GABAergic interneurons (Sik et al. 2000). Whole-cell patch-clamp recordings of recombinant ClC-2 channels harbouring either M200fsX231 or del74-117 mutations did not yield detectable chloride currents, compatible with a loss of function (Haug et al. 2003). There are several experimental evidences that ClC-2 channels are playing an important role in establishing and maintaining a low intracellular

chloride concentration, which is essential for neuronal inhibition mediated through $GABA_A$ receptors. Therefore, it is expected that loss of function mutations involving the ClC-2 channel will result in impaired chloride efflux, resulting in an abnormal intracellular accumulation of chloride. These mutant channels are thus expected to lower the transmembrane gradient that is essential for GABAergic inhibition, resulting in an increased excitability in neurons, which may lead to seizures. Interestingly, during brain development, as well as in pathological conditions (e.g. high frequency stimulation of pyramidal neurons of the hippocampus), such an intracellular accumulation of chloride may occur, resulting in reversal of the normal chloride gradient across the cell membrane (Kaila et al. 1997; Rivera et al. 1999). Under these conditions, $GABA_A$ receptors activity has been shown to exhibit reduced inhibitory, and eventually excitatory activity.

In contrast, the G715E mutation seems to cause the disease by a different mechanism. Indeed, patch-clamp recordings have shown that this mutation alters the chloride dependence of ClC-2 gating, resulting in an increase outward chloride current during repolarization. This gain of function may induce recurrent membrane depolarisation beyond action potential threshold, which can eventually lead to membrane depolarisation and neuronal hyperexcitability (Haug et al. 2003). More recently, two additional missense changes have been found in *CLCN2* in small nuclear families (D'Agostino et al. 2004). Functional studies of these variants are pending. Surprisingly, *CLCN2*-knockout mice do not exhibit epileptic phenotype (Bosl et al. 2001; Nehrke et al. 2002).

Overall, mutations in *CLCN2* have been shown to alter chloride gradient across the cellular membrane, which may eventually impair GABAergic neurotransmission in the brain. These findings are consistent with loss-of-function mutations found in at least

three different subunits of the $GABA_A$ for related epileptic syndromes. Taken together, these results suggest that impairment of the inhibition mechanisms mediated through the $GABA_A$ receptors is central in the pathophysiology of the classical IGE with autosomal dominant inheritance.

Impaired synaptic transmission caused by mutations in Synapsin 1

Synapsins are a family of neuron specific proteins that associate with synaptic vesicles (SV) (Greengard et al. 1993; Hilfiker et al. 1999). Three mammalian synapsin genes (*SYN1/2/3*) have been identified (Sudhof et al. 1989; Kao et al. 1998). All synapsins are highly expressed in the central nervous system, particularly in the temporal lobe and hippocampus (Matus-Leibovitch et al. 1997). Each of these genes is subject to alternative splicing of the last exon, resulting in at least nine isoforms (Fig. 1) (Porton et al. 1999).

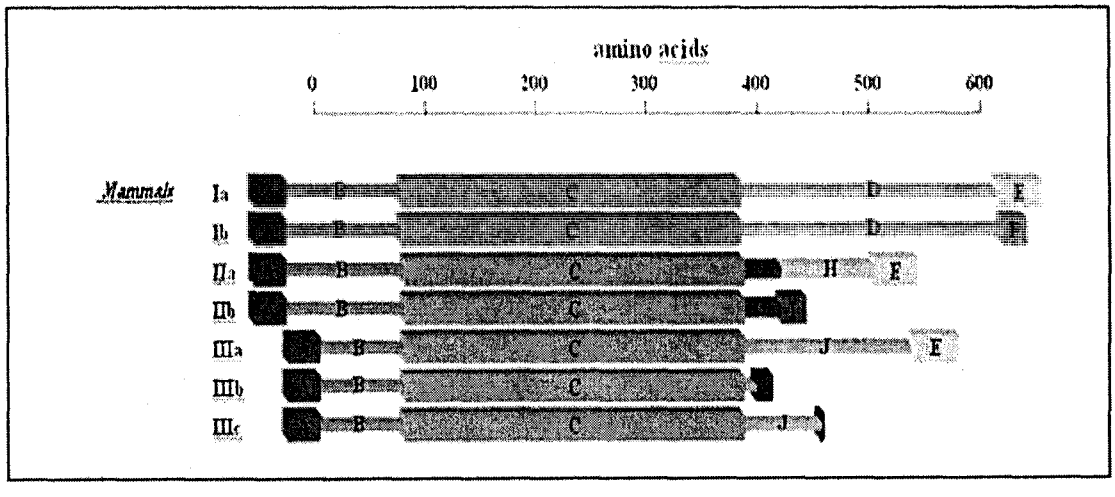


Figure 1. Different isoforms of synapsins (from Baldelli *et al.*, 2005).

The N-terminal portion of synapsins is well conserved (domains A, B, C), whereas the C-terminal portion is more variable (domains D, E, F, G, H, I, J). Synapsins bind, in a phosphorylation manner, to phospholipids and protein constituents of the synaptic vesicles, as well as to components of the cytoskeleton such as actin, tubulin, and spectrin (Baines and Bennett 1985; Baines and Bennett 1986; Bahler and Greengard 1987; Hilfiker et al. 1998). Synapsins interact with several protein kinases (Matsubara et al. 1996; Hilfiker et al. 1999) and other elements of cellular signaling pathways (Cousin et al. 2003; Tu et al. 2003). In particular, synapsins contain 11 minimal XPXXP motifs for interaction with the Src homology 3 (SH3) domains, and they have been shown to interact with an array of SH3 domains belonging to proteins involved in signal transduction, cytoskeleton, or endocytosis (Onofri et al. 2000; Giovedi et al. 2004). Disruption of synapsin in a variety of experimental models has been associated with: 1) increase in synaptic depression, indicating that synapsins are required to sustain neurotransmitter release during high levels of neuronal activity (Li et al. 1995; Pieribone et al. 1995; Rosahl et al. 1995); 2) decrease in the number of SV in the periphery of the active zone, suggesting that synapsins participate in transmitter release by regulating the reserve pool of SV (Baldelli et al. 2005); 3) perturbation of short-term synaptic plasticity, such as post-tetanic potentiation (Rosahl et al. 1993; Rosahl et al. 1995; Humeau et al. 2001), and 4) alteration in the post-docking step of the release process (Hilfiker et al. 1998; Humeau et al. 2001) (Fig. 2). Finally, there is growing evidences supporting that synapsins play an important role in the formation and maintenance of synaptic contacts, elongation of undifferentiated processes, and their differentiation into axon and dendrites (Ferreira and Rapoport 2002).

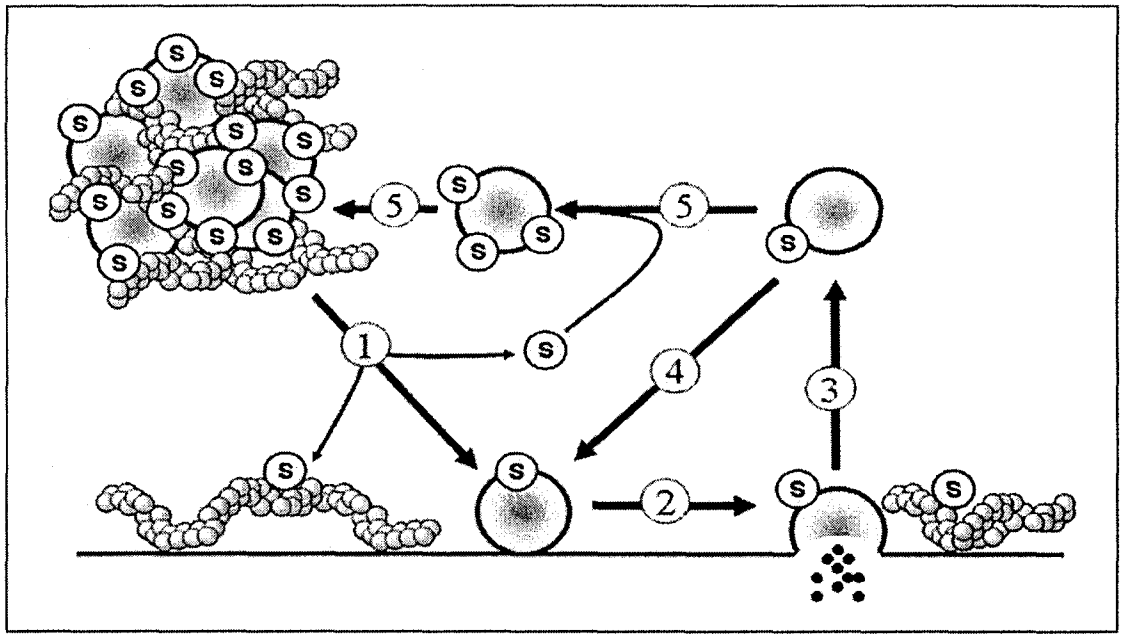


Figure 2. The effects of synapsins on synaptic vesicles release is a dynamic process. Dephosphorylated synapsins inhibit synaptic transmission and promote the recruitment of synaptic vesicles (SV) to the reserve pool by binding to both SV and actin. Upon activity dependant phosphorylation (step 1), synapsins dissociate from SV and actin and diffuse within the nerve preterminal regions of the axons. By acting at the active zone level, synapsins increase the rate of the post-docking events of priming and/or fusion, probably by interacting with the submembrane cystoskeleton meshwork or by removing the inhibitory action of Rab3 on the fusion process (from Baldelli *et al.*, 2005).

Two phosphorylation sites for Ca+/calmodulin-dependent protein kinase (CaMK) II reside in domain D of the synapsin 1 (Fig 1C, p.108). Inactivation of these CaMKII sites has been shown to impair the binding of *SYN1* with both actin and synaptic vesicles (Chi et al. 2001). In addition, COOH-terminal regions of *SYN1* bind to Src homology 3 domains (SH3) (Onofri et al. 2000), and Rab3 proteins (Giovedi et al. 2004), which are believed to play an important role in the activity-dependent regulation synaptic vesicle recycling. Truncation of *SYN1* caused by the Q555X mutation described in chapter three of this thesis is thus predicted to lead to dramatic changes in the binding properties of *SYN1*, which could impair neurotransmission. In a preliminary study, we have shown that knockdown of *Syn1* in zebrafish results in a severe motor deficit due to the loss of sustained synaptic transmission (Fig 2, p.110). This phenotype is partially rescued by overexpression of human *SYN1*, but not by a $SYN1_{Q555X}$ associated with TLE and language disorders, suggesting that this mutation is indeed pathogenic. Garcia *et al* previously reported a W356X mutation in *SYN1* (Garcia et al. 2004). In this latter family, affected males exhibit a mixture of neurobehavioural disturbance including epilepsy (n=8), learning disorder (n=3), episodic aggressive outbursts (n=3), and autism (n=1). As in the AB family described in this thesis, epilepsy with complex partial seizures appears to be the most robust phenotype in affected males. Taken together, these data confirm that nonsense mutations in *SYN1* predispose towards idiopathic partial epilepsy in males.

These observations are also consistent with the phenotype observed in *Syn1* KO mice. Each synapsin has been inactivated in mice (KO) (Baldelli et al. 2005). Despite the absence of gross defect in brain anatomy, *SYN1* and *SYN2* KO mice exhibit spontaneous

seizures, whereas *SYN3* KO mice does not show epilepsy. Crossing of these KO mice allow the study of double *SYN1/2* and triple *SYN1/2/3* KO mice, which exhibit the same epilepsy phenotype. The incidence of seizure is higher in *SYN2* KO mice, and is proportional to the number of inactivated synapsin. In *SYN1*, as well as in triple KO mice, impaired synaptic transmission is more striking in inhibitory synapses (GABAergic), compared to excitatory synapses (glutamatergic) (Terada et al. 1999; Gitler et al. 2004). This imbalance has been attributed to the fact that GABAergic interneurons experience high frequency firing rate, making them more vulnerable to depletion of the SV reserve pool. This relative loss of inhibitory transmission is believed to underlie the epilepsy phenotype observed in synapsins KO mice. Interestingly, in addition to the synapsins, disruption of several pre- and post-synaptic genes was also associated with epilepsy in mice (see p.27).

Molecular mechanisms in monogenic epilepsies: summary

With the identification of mutations in over 20 genes predisposing to idiopathic epilepsy in Humans (Meisler et al. 2001; Noebels 2003; Gourfinkel-An et al. 2004), as well as more than 121 genes leading to epilepsy-related phenotypes in mice (Noebels 2003), we can now try to better define which biological pathways are leading to the disease. With respect to these findings, inherited idiopathic epilepsies can be divided into three broad functional categories:

(1) *Primary defects of membrane excitability.* Examples in Humans include mutations in genes encoding for voltage-gated sodium (*SCN1A*, *SCN2A*, *SCN1B*) (Wallace et al. 1998; Escayg et al. 2000b), potassium (*KCNQ2*, *KCNQ3*, *KCNA1*, *KCNMA1*) (Biervert et al.

1998; Charlier et al. 1998; Singh et al. 1998; Zuberi et al. 1999; Eunson et al. 2000; Du et al. 2005), calcium (*CACNA1H*, *CACNB4*) (Escayg et al. 2000a; Chen et al. 2003; Heron et al. 2004; Khosravani et al. 2004; Khosravani et al. 2005; Vitko et al. 2005), and chloride channels (*CLCN2*) (Haug et al. 2003; D'Agostino et al. 2004). In addition, mutations in various ion channel genes may also cause epilepsy in mice (Noebels 2003);

(2) *Defects in synaptic signalling.* Examples in Humans are so far restricted to fast synaptic transmission, including mutations in *GABRA1, GABRG2*, and *GABRD*, encoding for the $\alpha 1$, $\gamma 2$, and δ subunit of the $GABA_A$ receptor (Baulac et al. 2001; Wallace et al. 2001a; Cossette et al. 2002; Harkin et al. 2002; Kananura et al. 2002; Dibbens et al. 2004), as well as in *CHRNA2*, *CHRNA4* and *CHRNB2*, encoding for the $\alpha 2$, $\alpha 4$ and $\beta 2$ subunits of the nicotinic acetylcholine receptor (Steinlein et al. 1995; De Fusco et al. 2000; Aridon et al. 2006). These mutations, respectively in ligand-gated chloride and sodium channels are additional examples of a channelopathy at the synaptic level. Mutations in *LGI1* are associated with impaired secretion of this glycoprotein and failure to bind its ligand ADAM22, which probably lead to abnormal AMPA receptor-mediated synaptic transmission in the hippocampus (Senechal et al. 2005; Fukata et al. 2006; Sirerol-Piquer et al. 2006). Finally, disruption of genes involved in GABAergic, glutamatergic, cholinergic and serotoninergic fast synaptic transmission has also been associated with epilepsy in mice (Noebels 2003);

(3) *Defects in neurotransmitter release machinery.* Defects in genes encoding for various proteins involved in the mobilization of neurotransmitter, as well as synaptic vesicle

trafficking and exocytosis, are also associated with epilepsy in mice. Examples of these mechanisms include: *SYN 1/2* knock-out, resulting in decreased size of the presynaptic vesicle pool, predominantly in inhibitory neurons (Rosahl et al. 1995; Terada et al. 1999); *Sv2A* knock-out, resulting in altered mobility of the releasable pool of synaptic vesicles (Crowder et al. 1999); *AP3δ* and *ZNT3* knock-out, resulting in incomplete synaptic vesicle assembly (Kantheti et al. 1998; Cole et al. 2000). In this thesis, we have shown that mutations in *SYN1* are associated with abnormal synaptic transmission and temporal lobe epilepsy in Humans, as previously suspected by another group of investigator (Garcia et al. 2004). Finally, in addition to genes involved in the presynaptic vesicle release apparatus, disruption of genes encoding for post-synaptic cytoskeletal scaffolds (e.g. ADAM22, PSD-95, stargazin) are also associated with epilepsy in mice (Letts et al. 1998; Sagane et al. 2005).

Additional mechanisms are most likely involved in idiopathic epilepsies, although these remain to be demonstrated. As an example, abnormal development of the temporal neocortex has been described in rare individuals with mutations in *LGI1* (Kobayashi et al. 2003). Interestingly, inactivation of *LGI1* has been found in glioblastoma, suggesting that *LGI1* may also act as a tumor suppressor gene (Chernova et al. 1998). Moreover, based on sequence homology, it has been suggested that *LGI1* could be involved in neurogenesis, axon guidance and synaptogenesis (Scheel et al. 2002). Perturbation of *LGI1* may thus be associated with either abnormal synaptic transmission in excitatory neurons, or abnormal development of neural networks, or both. Finally, autosomal dominant juvenile myoclonic epilepsy has been associated with mutations in *EFHC1* (Suzuki et al. 2004). This gene encodes a protein with an EF-hand motif involved in

calcium binding, but its exact function remains unknown. Overexpression of *EFHC1* in mouse hippocampal primary culture neurons induced apoptosis that was significantly lowered by the mutations found in JME families (Suzuki et al. 2004). It is possible that *EFHC1* mutations result in insufficient apoptotic shedding of unnecessary neurons during development, and produce an imperfect, overpopulated and epileptogenic, cerebral network. This latter hypothesis is supported by pathological and imaging studies, which have shown thickened cerebral cortex, and cortical dysplasia in JME patients (Turnbull et al. 2005). Further work will be needed in order to validate these latter mechanims. As observed in individuals with mutation in *LGI1*, abnormal membrane excitability or altered synaptic transmission observed in other monogenic epilepsies may ultimately pertub the normal wiring of the brain, which may not be easily detected on conventional brain imaging. Presumably, these abnormal networks would be more susceptible to abnormal firing and dysfunction, potentially underlying both epilepsy and associated cognitive impairment.

BIBLIOGRAPHY

Aghakhani Y, Bagshaw AP, Benar CG, Hawco C, Andermann F, Dubeau F, Gotman J (2004) fMRI activation during spike and wave discharges in idiopathic generalized epilepsy. Brain 127:1127-1144

Aicardi J (1986) Epilepsy in Children. Raven Press, New York

Aldenkamp AP (1997) Effect of seizures and epileptiform discharges on cognitive function. Epilepsia 38 Suppl 1:S52-55

Aldenkamp AP, Bodde N (2005) Behaviour, cognition and epilepsy. Acta Neurol Scand Suppl 182:19-25

Alekov A, Rahman MM, Mitrovic N, Lehmann-Horn F, Lerche H (2000) A sodium channel mutation causing epilepsy in man exhibits subtle defects in fast inactivation and activation in vitro. J Physiol 529:533-539

Alekov AK, Rahman MM, Mitrovic N, Lehmann-Horn F, Lerche H (2001) Enhanced inactivation and acceleration of activation of the sodium channel associated with epilepsy in man. Eur J Neurosci 13:2171-2176

Alonso A, Llinas RR (1989) Subthreshold Na+-dependent theta-like rhythmicity in stellate cells of entorhinal cortex layer II. Nature 342:175-177

Andermann F, Berkovic SF (2001) Idiopathic generalized epilepsy with generalized and other seizures in adolescence. Epilepsia 42:317-320

Annegers JF, Hauser WA, Anderson VE, Kurland LT (1982) The risks of seizure disorders among relatives of patients with childhood onset epilepsy. Neurology 32:174-179

Aridon P, Marini C, Di Resta C, Brilli E, De Fusco M, Politi F, Parrini E, Manfredi I, Pisano T, Pruna D, Curia G, Cianchetti C, Pasqualetti M, Becchetti A, Guerrini R, Casari G (2006) Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. Am J Hum Genet 79:342-350

Audenaert D, Claes L, Ceulemans B, Lofgren A, Van Broeckhoven C, De Jonghe P (2003) A deletion in SCN1B is associated with febrile seizures and early-onset absence epilepsy. Neurology 61:854-856

Auld VJ, Goldin AL, Krafte DS, Marshall J, Dunn JM, Catterall WA, Lester HA, Davidson N, Dunn RJ (1988) A rat brain Na+ channel alpha subunit with novel gating properties. Neuron 1:449-461

Avanzini G, al. (1997) Animal models of localization-related epilepsy. In: Engel Jr J, Pedley TA (eds) Epilepsy: A comprehensive Textbook. Lippincott-Raven, New York, pp 427-442

Avoli M, Louvel J, Pumain R, Kohling R (2005) Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol 77:166-200

Bahler M, Greengard P (1987) Synapsin I bundles F-actin in a phosphorylation-dependent manner. Nature 326:704-707

Baines AJ, Bennett V (1985) Synapsin I is a spectrin-binding protein immunologically related to erythrocyte protein 4.1. Nature 315:410-413

Baines AJ, Bennett V (1986) Synapsin I is a microtubule-bundling protein. Nature 319:145-147

Baldelli P, Fassio A, Corradi A, Cremona O, Valtorta F, Benfenati F (2005) Synapsins and neuroexocytosis: recent views from functional studies on synapsin null mutant mice. Arch Ital Biol 143:113-126

Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E (2001) First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 28:46-48

Baykan B, Madia F, Bebek N, Gianotti S, Guney AI, Cine N, Bianchi A, Gokyigit A, Zara F (2004) Autosomal recessive idiopathic epilepsy in an inbred family from Turkey: identification of a putative locus on chromosome 9q32-33. Epilepsia 45:479-487

Beaumanoir A, Blume W (2002) The Lennox-Gastaut syndrome. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari CA, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 113-136

Becker AJ, Chen J, Zien A, Sochivko D, Normann S, Schramm J, Elger CE, Wiestler OD, Blumcke I (2003) Correlated stage- and subfield-associated hippocampal gene expression patterns in experimental and human temporal lobe epilepsy. Eur J Neurosci 18:2792-2802

Benardo LS, Prince DA (1982) Cholinergic excitation of mammalian hippocampal pyramidal cells. Brain Res 249:315-331

Bennett FE (1953) Intracarotid and intravertebral metrazol in petit mal epilepsy. Neurology 3:668-673

Bennett PB, Yazawa K, Makita N, George AL, Jr. (1995) Molecular mechanism for an inherited cardiac arrhythmia. Nature 376:683-685

Berkovic SF, Andermann F, Andermann E, Gloor P (1987) Concepts of absence epilepsies: discrete syndromes or biological continuum? Neurology 37:993-1000

Berkovic SF, Heron SE, Giordano L, Marini C, Guerrini R, Kaplan RE, Gambardella A, Steinlein OK, Grinton BE, Dean JT, Bordo L, Hodgson BL, Yamamoto T, Mulley JC, Zara F, Scheffer IE (2004) Benign familial neonatal-infantile seizures: characterization of a new sodium channelopathy. Ann Neurol 55:550-557

Berkovic SF, Howell RA, Hay DA, Hopper JL (1998) Epilepsies in twins: genetics of the major epilepsy syndromes. Ann Neurol 43:435-445

Berkovic SF, McIntosh A, Howell RA, Mitchell A, Sheffield LJ, Hopper JL (1996) Familial temporal lobe epilepsy: a common disorder identified in twins. Ann Neurol 40:227-235

Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK (1998) A potassium channel mutation in neonatal human epilepsy. Science 279:403-406

Bishop DV, Adams C (1990) A prospective study of the relationship between specific language impairment, phonological disorders and reading retardation. J Child Psychol Psychiatry 31:1027-1050

Blume WT, Luders HO, Mizrahi E, Tassinari C, van Emde Boas W, Engel J, Jr. (2001) Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology. Epilepsia 42:1212-1218

Bosl MR, Stein V, Hubner C, Zdebik AA, Jordt SE, Mukhopadhyay AK, Davidoff MS, Holstein AF, Jentsch TJ (2001) Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon ClC-2 Cl(-) channel disruption. Embo J 20:1289-1299

Cannon SC, Strittmatter SM (1993) Functional expression of sodium channel mutations identified in families with periodic paralysis. Neuron 10:317-326

Cassidy G, Corbett J (1997) Learning disorders. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 2053-2063

Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L (1991) Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia 32:778-782

Chahine M, George AL, Jr., Zhou M, Ji S, Sun W, Barchi RL, Horn R (1994) Sodium channel mutations in paramyotonia congenita uncouple inactivation from activation. Neuron 12:281-294

Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M (1998) A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat Genet 18:53-55

Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, Liu X, Jiang Y, Bao X, Yao Z, Ding K, Lo WH, Qiang B, Chan P, Shen Y, Wu X (2003) Association between genetic variation of CACNA1H and childhood absence epilepsy. Ann Neurol 54:239-243

Chernova OB, Somerville RP, Cowell JK (1998) A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. Oncogene 17:2873-2881

Chi P, Greengard P, Ryan TA (2001) Synapsin dispersion and reclustering during synaptic activity. Nat Neurosci 4:1187-1193

Chioza B, Wilkie H, Nashef L, Blower J, McCormick D, Sham P, Asherson P, Makoff AJ (2001) Association between the alpha(1a) calcium channel gene CACNA1A and idiopathic generalized epilepsy. Neurology 56:1245-1246

Claes L, Audenaert D, Deprez L, Van Paesschen W, Depondt C, Goossens D, Del-Favero J, Van Broeckhoven C, De Jonghe P (2004) Novel locus on chromosome 12q22-q23.3 responsible for familial temporal lobe epilepsy associated with febrile seizures. J Med Genet 41:710-714

Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P (2001) De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet 68:1327-1332

Colbert CM, Magee JC, Hoffman DA, Johnston D (1997) Slow recovery from inactivation of Na+ channels underlies the activity-dependent attenuation of dendritic action potentials in hippocampal CA1 pyramidal neurons. J Neurosci 17:6512-6521

Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD (2000) Seizures and neuronal damage in mice lacking vesicular zinc. Epilepsy Res 39:153-169

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J Neurosci 22:5572-5580

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, Holmans P, Owen MJ, O'Donovan MC, Williams J (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76:581-591

Corey LA, Berg K, Pellock JM, Solaas MH, Nance WE, DeLorenzo RJ (1991) The occurrence of epilepsy and febrile seizures in Virginian and Norwegian twins. Neurology 41:1433-1436

Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, Rouleau GA (2002) Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet 31:184-189

Cossette P, Loukas A, Lafreniere RG, Rochefort D, Harvey-Girard E, Ragsdale DS, Dunn RJ, Rouleau GA (2003) Functional characterization of the D188V mutation in neuronal voltage-gated sodium channel causing generalized epilepsy with febrile seizures plus (GEFS). Epilepsy Res 53:107-117

Cousin MA, Malladi CS, Tan TC, Raymond CR, Smillie KJ, Robinson PJ (2003) Synapsin I-associated phosphatidylinositol 3-kinase mediates synaptic vesicle delivery to the readily releasable pool. J Biol Chem 278:29065-29071

Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C, Bajjalieh SM (1999) Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). Proc Natl Acad Sci U S A 96:15268-15273

Culiat CT, Stubbs LJ, Montgomery CS, Russell LB, Rinchik EM (1994) Phenotypic consequences of deletion of the gamma 3, alpha 5, or beta 3 subunit of the type A gamma-aminobutyric acid receptor in mice. Proc Natl Acad Sci U S A 91:2815-2818

D'Agostino D, Bertelli M, Gallo S, Cecchin S, Albiero E, Garofalo PG, Gambardella A, St Hilaire JM, Kwiecinski H, Andermann E, Pandolfo M (2004) Mutations and polymorphisms of the CLCN2 gene in idiopathic epilepsy. Neurology 63:1500-1502

Dalla Bernardina B, Sgro V, Fejerman N (2002) Epilepsy with centro-temporal spikes and related syndromes. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari CA, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 181-202

De Fusco M, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, Ballabio A, Wanke E, Casari G (2000) The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet 26:275-276

DeFries JC, Fulker DW, LaBuda MC (1987) Evidence for a genetic aetiology in reading disability of twins. Nature 329:537-539

Delgado-Escueta AV, Enrile-Bacsal F (1984) Juvenile myoclonic epilepsy of Janz. Neurology 34:285-294

DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW (1998) Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. J Neurosci 18:8505-8514

Depondt C, Van Paesschen W, Matthijs G, Legius E, Martens K, Demaerel P, Wilms G (2002) Familial temporal lobe epilepsy with febrile seizures. Neurology 58:1429-1433

Deprez L, Claes LR, Claeys KG, Audenaert D, Van Dyck T, Goossens D, Van Paesschen W, Del-Favero J, Van Broeckhoven C, De Jonghe P (2006) Genome-wide linkage of

febrile seizures and epilepsy to the FEB4 locus at 5q14.3-q23.1 and no MASS1 mutation. Hum Genet 118:618-625

Desguerre I, Chiron C, Loiseau J, al (1994) Epidemiology of idiopathic generalized epilepsies. In: Malafosse A, Genton P, Hirsch E, Maresceaux C, Broglin D, Bernasconi R (eds) Idiopathic generalized epilepsies. John Libbey, London, pp 19-25

Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC (2004) GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet 13:1315-1319

Dichter MA (1997) Overview: The neurobiology of epilepsy. In: Engel Jr J, Pedley TA (eds) Epilepsy: A comprehensive textbook. Lippincott-Raven, New York, pp 233-235

Doose H, Gerken H (1973) On the genetics of EEG-anomalies in childhood. IV. Photoconvulsive reaction. Neuropaediatrie 4:162-171

Drapeau P, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Brustein E (2002) Development of the locomotor network in zebrafish. Prog Neurobiol 68:85-111

Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O (2002) Severe myoclonic epilepsy in infancy (Dravet syndrome). In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 81-104

Du F, Whetsell WO, Jr., Abou-Khalil B, Blumenkopf B, Lothman EW, Schwarcz R (1993) Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. Epilepsy Res 16:223-233

Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK (2005) Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. Nat Genet 37:733-738

Duenas AM, Goold R, Giunti P (2006) Molecular pathogenesis of spinocerebellar ataxias. Brain 129:1357-1370

Dulac O, Tuxhorn I (2002) Infantile spasms and West syndrome. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 47-64

Eadie MJ, Bladin PF (2001) A disease once sacred. John Libbey, Eastleigh

Eaves IA, Merriman TR, Barber RA, Nutland S, Tuomilehto-Wolf E, Tuomilehto J, Cucca F, Todd JA (2000) The genetically isolated populations of Finland and sardinia may not be a panacea for linkage disequilibrium mapping of common disease genes. Nat Genet 25:320-323

Eeg-Olofsson O, Petersen I, Sellden U (1971) The development of the electroencephalogram in normal children from the age of 1 through 15 years. Paroxysmal activity. Neuropaediatrie 2:375-404

Elmslie FV, Rees M, Williamson MP, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Chadwick D, Richens A, Covanis A, Santos M, Arzimanoglou A, Panayiotopoulos CP, Curtis D, Whitehouse WP, Gardiner RM (1997) Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. Hum Mol Genet 6:1329-1334

Elmslie FV, Williamson MP, Rees M, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Richens A, Chadwick D, Whitehouse WP, Gardiner RM (1996) Linkage analysis of juvenile myoclonic epilepsy and microsatellite loci spanning 61 cM of human chromosome 6p in 19 nuclear pedigrees provides no evidence for a susceptibility locus in this region. Am J Hum Genet 59:653-663

Emery AE (2002) The muscular dystrophies. Lancet 359:687-695

Engel J, Jr. (1998) Classifications of the International League Against Epilepsy: time for reappraisal. Epilepsia 39:1014-1017

Engel J, Jr. (2001) A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 42:796-803

Engel J, Jr. (2006) Report of the ILAE classification core group. Epilepsia 47:1558-1568

Engel J, Jr., Henry TR, Risinger MW, Mazziotta JC, Sutherling WW, Levesque MF, Phelps ME (1990) Presurgical evaluation for partial epilepsy: relative contributions of chronic depth-electrode recordings versus FDG-PET and scalp-sphenoidal ictal EEG. Neurology 40:1670-1677

Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindvall O (1991) Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 7:165-176

Escayg A, De Waard M, Lee DD, Bichet D, Wolf P, Mayer T, Johnston J, Baloh R, Sander T, Meisler MH (2000a) Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am J Hum Genet 66:1531-1539

Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A (2000b) Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet 24:343-345

Eunson LH, Rea R, Zuberi SM, Youroukos S, Panayiotopoulos CP, Liguori R, Avoni P, McWilliam RC, Stephenson JB, Hanna MG, Kullmann DM, Spauschus A (2000) Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. Ann Neurol 48:647-656

Ferreira A, Rapoport M (2002) The synapsins: beyond the regulation of neurotransmitter release. Cell Mol Life Sci 59:589-595

Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 46:470-472

Fisher SE, DeFries JC (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. Nat Rev Neurosci 3:767-780

Folstein SE, Santangelo SL, Gilman SE, Piven J, Landa R, Lainhart J, Hein J, Wzorek M (1999) Predictors of cognitive test patterns in autism families. J Child Psychol Psychiatry 40:1117-1128

Fong GC, Shah PU, Gee MN, Serratosa JM, Castroviejo IP, Khan S, Ravat SH, Mani J, Huang Y, Zhao HZ, Medina MT, Treiman LJ, Pineda G, Delgado-Escueta AV (1998) Childhood absence epilepsy with tonic-clonic seizures and electroencephalogram 3-4-

Hz spike and multispike-slow wave complexes: linkage to chromosome 8q24. Am J Hum Genet 63:1117-1129

Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M (2006) Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. Science 313:1792-1795

Gall CM, Isackson PJ (1989) Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. Science 245:758-761

Garcia CC, Blair HJ, Seager M, Coulthard A, Tennant S, Buddles M, Curtis A, Goodship JA (2004) Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. J Med Genet 41:183-186

Genton P, Salas-Puig X, Tunon A, Lahoz CH, Del Socorro M, Sanchez G (1994). In: Idiopathic Generalized Epilepsies: Clinical, Experimental and Genetics Aspects. John Libbey, London, pp 253-265

Gingrich JC, Boehrer DM, Garnes JA, Johnson W, Wong BS, Bergmann A, Eveleth GG, Langlois RG, Carrano AV (1996) Construction and characterization of human chromosome 2-specific cosmid, fosmid, and PAC clone libraries. Genomics 32:65-74

Giovedi S, Darchen F, Valtorta F, Greengard P, Benfenati F (2004) Synapsin is a novel Rab3 effector protein on small synaptic vesicles. II. Functional effects of the Rab3A-synapsin I interaction. J Biol Chem 279:43769-43779

Gitler D, Takagishi Y, Feng J, Ren Y, Rodriguiz RM, Wetsel WC, Greengard P, Augustine GJ (2004) Different presynaptic roles of synapsins at excitatory and inhibitory synapses. J Neurosci 24:11368-11380

Gloor P (1968) Generalized cortico-reticular epilepsies. Some considerations on the pathophysiology of generalized bilaterally synchronous spike and wave discharge. Epilepsia 9:249-263

Gloor P (1997) The temporal lobe and limbic system. Oxford University Press, Oxford

Goddard GV (1967) Development of epileptic seizures through brain stimulation at low intensity. Nature 214:1020-1021

Goddard GV, McIntyre DC, Leech CK (1969) A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 25:295-330

Goldensohn ES (1997) Historial perspectives. In: Engel Jr J, Pedley TA (eds) Epilepsy: A comprehensive textbook. Lippincott-Raven, New York, pp 15-39

Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA (2000) Nomenclature of voltage-gated sodium channels. Neuron 28:365-368

Gourfinkel-An I, Baulac S, Nabbout R, Ruberg M, Baulac M, Brice A, LeGuern E (2004) Monogenic idiopathic epilepsies. Lancet Neurol 3:209-218

Greenberg DA, Cayanis E, Strug L, Marathe S, Durner M, Pal DK, Alvin GB, Klotz I, Dicker E, Shinnar S, Bromfield EB, Resor S, Cohen J, Moshe SL, Harden C, Kang H (2005) Malic enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. Am J Hum Genet 76:139-146

Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman L, Maldonado HM, Park MS, Terasaki PI (1988) Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. Am J Med Genet 31:185-192

Greenberg DA, Durner M, Keddache M, Shinnar S, Resor SR, Moshe SL, Rosenbaum D, Cohen J, Harden C, Kang H, Wallace S, Luciano D, Ballaban-Gil K, Tomasini L, Zhou G, Klotz I, Dicker E (2000) Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. Am J Hum Genet 66:508-516

Greengard P, Valtorta F, Czernik AJ, Benfenati F (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. Science 259:780-785

Guerrini R, Bonanni P, Nardocci N, Parmeggiani L, Piccirilli M, De Fusco M, Aridon P, Ballabio A, Carrozzo R, Casari G (1999) Autosomal recessive rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp: delineation of the syndrome and gene mapping to chromosome 16p12-11.2. Ann Neurol 45:344-352

Guerrini R, Bonanni P, Patrignani A, Brown P, Parmeggiani L, Grosse P, Brovedani P, Moro F, Aridon P, Carrozzo R, Casari G (2001) Autosomal dominant cortical myoclonus and epilepsy (ADCME) with complex partial and generalized seizures: A newly recognized epilepsy syndrome with linkage to chromosome 2p11.1-q12.2. Brain 124:2459-2475

Guerrini R, Parmeggiani L, Kaminska A, Dulac O (2002) Myoclonic astatic epilepsy. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 105-112

Guipponi M, Rivier F, Vigevano F, Beck C, Crespel A, Echenne B, Lucchini P, Sebastianelli R, Baldy-Moulinier M, Malafosse A (1997) Linkage mapping of benign familial infantile convulsions (BFIC) to chromosome 19q. Hum Mol Genet 6:473-477

Gunther U, Benson J, Benke D, Fritschy JM, Reyes G, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y, et al. (1995) Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 92:7749-7753

Habib M (2000) The neurological basis of developmental dyslexia: an overview and working hypothesis. Brain 123:2373-2399

Halliwell JV (1986) M-current in human neocortical neurones. Neurosci Lett 67:1-6

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85-100

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, Kere J (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1:e50

Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S (2002) Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. Am J Hum Genet 70:530-536

Haug K, Warnstedt M, Alekov AK, Sander T, Ramirez A, Poser B, Maljevic S, Hebeisen S, Kubisch C, Rebstock J, Horvath S, Hallmann K, Dullinger JS, Rau B, Haverkamp F, Beyenburg S, Schulz H, Janz D, Giese B, Muller-Newen G, Propping P, Elger CE, Fahlke C, Lerche H, Heils A (2003) Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. Nat Genet 33:527-532

Hauser WA (1997) Incidence and prevalence. In: Engel Jr J, Pedley TA (eds) Epilepsy: A comprehensive textbook. Lippincott-Raven, New York, pp 47-57

Hauser WA, Annegers JF, Anderson VE, Kurland LT (1985) The risk of seizure disorders among relatives of children with febrile convulsions. Neurology 35:1268-1273

Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, Shevell M, Mercho S, Seni MH, Guiot MC, Mulley JC, Berkovic SF, Scheffer IE (2002) Sodium-channel defects in benign familial neonatal-infantile seizures. Lancet 360:851-852

Heron SE, Phillips HA, Mulley JC, Mazarib A, Neufeld MY, Berkovic SF, Scheffer IE (2004) Genetic variation of CACNA1H in idiopathic generalized epilepsy. Ann Neurol 55:595-596

Hilfiker S, Pieribone VA, Czernik AJ, Kao HT, Augustine GJ, Greengard P (1999) Synapsins as regulators of neurotransmitter release. Philos Trans R Soc Lond B Biol Sci 354:269-279

Hilfiker S, Schweizer FE, Kao HT, Czernik AJ, Greengard P, Augustine GJ (1998) Two sites of action for synapsin domain E in regulating neurotransmitter release. Nat Neurosci 1:29-35

Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CE, Korpi ER, Makela R, Brilliant MH, Hagiwara N, Ferguson C, Snyder K, Olsen RW (1997) Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. Proc Natl Acad Sci U S A 94:4143-4148

Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV (1990) Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. J Neurosci 10:267-282

Humeau Y, Doussau F, Vitiello F, Greengard P, Benfenati F, Poulain B (2001) Synapsin controls both reserve and releasable synaptic vesicle pools during neuronal activity and short-term plasticity in Aplysia. J Neurosci 21:4195-4206

ICD-10 (1992) International statistical classification of diseases and related health problems (10^{th} edn). World Health Organization, Geneva

ILAE (1993) Concordance of clinical forms of epilepsy in families with several affected members. Italian League Against Epilepsy Genetic Collaborative Group. Epilepsia 34:819-826

ILEA (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 22:489-501

ILEA (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 30:389-399

Inouye E (1960) Observations on forty twin index cases with chronic epilepsy and their co-twins. J Nerv Ment Dis 130:401-416

Isom LL (2001) Sodium channel beta subunits: anything but auxiliary. Neuroscientist 7:42-54

Ito M, Ohmori I, Nakahori T, Ouchida M, Ohtsuka Y (2005) Mutation screen of GABRA1, GABRB2 and GABRG2 genes in Japanese patients with absence seizures. Neurosci Lett 383:220-224

Jackson GD, McIntosh AM, Briellmann RS, Berkovic SF (1998) Hippocampal sclerosis studied in identical twins. Neurology 51:78-84

Jackson JH (1870) A study of convulsions. In: Transactions of the St-Andrews Medical Graduates Association. Vol 3, pp 162-204. Reprinted in: Taylor JE ed. (1958) Selected writings of John Hughlings Jackson (vol 1), Staples Press, London

Jackson JH (1887) Remarks on evolution and dissolution of the nervous system. In: Journal of mental science. Reprinted in: Taylor JE ed. (1958) Selected writings of John Hughlings Jackson (vol 2), Staples Press, London

Jain S, Padma MV, Puri A, Jyoti, Maheshwari MC (1997) Occurrence of epilepsies in family members of Indian probands with different epileptic syndromes. Epilepsia 38:237-244

Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet 34:27-29

Janz D, Durner M (1997) Juvenile myoclonic epilepsy. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive Textbook. Lippincott-Raven, New York, pp 2389-2400

Jasper HH, Kershmann J (1941) Electroencephalographic classification of the epilepsies. Arch Neurol Psychiatry 45:903-943

Johnson EW, Dubovsky J, Rich SS, O'Donovan CA, Orr HT, Anderson VE, Gil-Nagel A, Ahmann P, Dokken CG, Schneider DT, Weber JL (1998) Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the Midwest. Hum Mol Genet 7:63-67

Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, Hanna MG (2001) Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. Lancet 358:801-807

Jung HY, Mickus T, Spruston N (1997) Prolonged sodium channel inactivation contributes to dendritic action potential attenuation in hippocampal pyramidal neurons. J Neurosci 17:6639-6646

Kaila K, Lamsa K, Smirnov S, Taira T, Voipio J (1997) Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K+ transient. J Neurosci 17:7662-7672

Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, Choi C, Morozov P, Das K, Teplitskaya E, Yu A, Cayanis E, Penchaszadeh G, Kottmann AH, Pedley TA, Hauser WA, Ottman R, Gilliam TC (2002) Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet 30:335-341

Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, Rebstock J, Heils A, Steinlein OK (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 59:1137-1141

Kang JQ, Macdonald RL (2004) The GABAA receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. J Neurosci 24:8672-8677

Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, Kapfhamer D, Sufalko D, Robinson MS, Noebels JL, Burmeister M (1998) Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. Neuron 21:111-122

Kao HT, Porton B, Czernik AJ, Feng J, Yiu G, Haring M, Benfenati F, Greengard P (1998) A third member of the synapsin gene family. Proc Natl Acad Sci U S A 95:4667-4672

Kazen-Gillespie KA, Ragsdale DS, D'Andrea MR, Mattei LN, Rogers KE, Isom LL (2000) Cloning, localization, and functional expression of sodium channel beta1A subunits. J Biol Chem 275:1079-1088

Khosravani H, Altier C, Simms B, Hamming KS, Snutch TP, Mezeyova J, McRory JE, Zamponi GW (2004) Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. J Biol Chem 279:9681-9684

Khosravani H, Bladen C, Parker DB, Snutch TP, McRory JE, Zamponi GW (2005) Effects of Cav3.2 channel mutations linked to idiopathic generalized epilepsy. Ann Neurol 57:745-749

Kinton L, Johnson MR, Smith SJ, Farrell F, Stevens J, Rance JB, Claudino AM, Duncan JS, Davis MB, Wood NW, Sander JW (2002) Partial epilepsy with pericentral spikes:

a new familial epilepsy syndrome with evidence for linkage to chromosome 4p15. Ann Neurol 51:740-749

Kjelgaard MM, Tager-Flusberg H (2001) An Investigation of Language Impairment in Autism: Implications for Genetic Subgroups. Lang Cogn Process 16:287-308

Knowles WD, Awad IA, Nayel MH (1992) Differences of in vitro electrophysiology of hippocampal neurons from epileptic patients with mesiotemporal sclerosis versus structural lesions. Epilepsia 33:601-609

Kobayashi E, Santos NF, Torres FR, Secolin R, Sardinha LA, Lopez-Cendes I, Cendes F (2003) Magnetic resonance imaging abnormalities in familial temporal lobe epilepsy with auditory auras. Arch Neurol 60:1546-1551

Kralic JE, Korpi ER, O'Buckley TK, Homanics GE, Morrow AL (2002) Molecular and pharmacological characterization of GABA(A) receptor alpha1 subunit knockout mice. J Pharmacol Exp Ther 302:1037-1045

Krampfl K, Maljevic S, Cossette P, Ziegler E, Rouleau GA, Lerche H, Bufler J (2005) Molecular analysis of the A322D mutation in the GABA receptor alpha-subunit causing juvenile myoclonic epilepsy. Eur J Neurosci 22:10-20

Kraus JE, Yeh GC, Bonhaus DW, Nadler JV, McNamara JO (1994) Kindling induces the long-lasting expression of a novel population of NMDA receptors in hippocampal region CA3. J Neurosci 14:4196-4205

Kwan P, Sills GJ, Brodie MJ (2001) The mechanisms of action of commonly used antiepileptic drugs. Pharmacol Ther 90:21-34

Landau WM, Kleffner FR (1957) Syndrome of acquired aphasia with convulsive disorder in children. Neurology 7:523-530

Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460-465

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci U S A 81:3443-3446

Lennox WG (1951) The heredity of epilepsy as told by relatives and twins. J Am Med Assoc 146:529-536

Letts VA, Felix R, Biddlecome GH, Arikkath J, Mahaffey CL, Valenzuela A, Bartlett FS, 2nd, Mori Y, Campbell KP, Frankel WN (1998) The mouse stargazer gene encodes a neuronal Ca2+-channel gamma subunit. Nat Genet 19:340-347

Lewis DV (1999) Febrile convulsions and mesial temporal sclerosis. Curr Opin Neurol 12:197-201

Li L, Chin LS, Shupliakov O, Brodin L, Sihra TS, Hvalby O, Jensen V, Zheng D, McNamara JO, Greengard P, et al. (1995) Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice. Proc Natl Acad Sci U S A 92:9235-9239

Lohi H, Chan EM, Scherer SW, Minassian BA (2006) On the road to tractability: the current biochemical understanding of progressive myoclonus epilepsies. Adv Neurol 97:399-415

Loiseau J (1997) Seizure precipitants. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 93-97

Loiseau P, Panayiotopoulos CP, Hirsch E (2002) Chidhood absence epilepsy and related syndromes. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds)

Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 285-304

Lopes-Cendes I, Scheffer IE, Berkovic SF, Rousseau M, Andermann E, Rouleau GA (2000) A new locus for generalized epilepsy with febrile seizures plus maps to chromosome 2. Am J Hum Genet 66:698-701

Lorenzon NM, Foehring RC (1992) Relationship between repetitive firing and afterhyperpolarizations in human neocortical neurons. J Neurophysiol 67:350-363

Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, Devinsky O, George AL, Jr. (2003) Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. J Neurosci 23:11289-11295

Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL, Jr. (2002) Molecular basis of an inherited epilepsy. Neuron 34:877-884

Lothman EW, Collins RC, Ferrendelli JA (1981) Kainic acid-induced limbic seizures: electrophysiologic studies. Neurology 31:806-812

Lu WY, Xiong ZG, Orser BA, MacDonald JF (1998) Multiple sites of action of neomycin, Mg2+ and spermine on the NMDA receptors of rat hippocampal CA1 pyramidal neurones. J Physiol 512:29-46

Ma S, Blair MA, Abou-Khalil B, Lagrange AH, Gurnett CA, Hedera P (2006) Mutations in the GABRA1 and EFHC1 genes are rare in familial juvenile myoclonic epilepsy. Epilepsy Res 71:129-134

Madia F, Striano P, Gennaro E, Malacarne M, Paravidino R, Biancheri R, Budetta M, Cilio MR, Gaggero R, Pierluigi M, Minetti C, Zara F (2006) Cryptic chromosome

deletions involving SCN1A in severe myoclonic epilepsy of infancy. Neurology 67:1230-1235

Maljevic S, Krampfl K, Cobilanschi J, Tilgen N, Beyer S, Weber YG, Schlesinger F, Ursu D, Melzer W, Cossette P, Bufler J, Lerche H, Heils A (2006) A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. Ann Neurol 59:983-987

Mandelbaum DE, Burack GD (1997) The effect of seizure type and medication on cognitive and behavioral functioning in children with idiopathic epilepsy. Dev Med Child Neurol 39:731-735

Marini C, Scheffer IE, Crossland KM, Grinton BE, Phillips FL, McMahon JM, Turner SJ, Dean JT, Kivity S, Mazarib A, Neufeld MY, Korczyn AD, Harkin LA, Dibbens LM, Wallace RH, Mulley JC, Berkovic SF (2004) Genetic architecture of idiopathic generalized epilepsy: clinical genetic analysis of 55 multiplex families. Epilepsia 45:467-478

Marshall AG, Hutchinson EO, Honisett J (1962) Heredity in common diseases. A retrospective survey of twins in a hospital population. Br Med J 1:1-6

Martin JH (1991) The collective electrical behaviour of cortical neurons: the electroencephalogram and the mechanisms of epilepsy. In: Kandel ER, Schwartz JH, Jessel TM (eds) Principles of neural sciences. Elsevier Science, Amsterdam, pp 777-791

Matsubara M, Kusubata M, Ishiguro K, Uchida T, Titani K, Taniguchi H (1996) Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions. J Biol Chem 271:21108-21113

Matus-Leibovitch N, Nevo I, Vogel Z (1997) Differential distribution of synapsin IIa and IIb mRNAs in various brain structures and the effect of chronic morphine administration on the regional expression of these isoforms. Brain Res Mol Brain Res 45:301-316

McNamara JO (1994) Cellular and molecular basis of epilepsy. J Neurosci 14:3413-3425

McNamara JO, Wada JA (1997) Kindling model. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 419-425

Meadows LS, Malhotra J, Loukas A, Thyagarajan V, Kazen-Gillespie KA, Koopman MC, Kriegler S, Isom LL, Ragsdale DS (2002) Functional and biochemical analysis of a sodium channel beta1 subunit mutation responsible for generalized epilepsy with febrile seizures plus type 1. J Neurosci 22:10699-10709

Meeren H, van Luijtelaar G, Lopes da Silva F, Coenen A (2005) Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory. Arch Neurol 62:371-376

Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH (2002) Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J Neurosci 22:1480-1495

Meisler MH, Kearney J, Ottman R, Escayg A (2001) Identification of epilepsy genes in human and mouse. Annu Rev Genet 35:567-588

Metrakos K, Metrakos JD (1961) Genetics of convulsive disorders II. Genetic and electroencephalographic studies in centrencephalic epilepsy. Neurology 11:474-483

Mikami M, Yasuda T, Terao A, Nakamura M, Ueno S, Tanabe H, Tanaka T, Onuma T, Goto Y, Kaneko S, Sano A (1999) Localization of a gene for benign adult familial myoclonic epilepsy to chromosome 8q23.3-q24.1. Am J Hum Genet 65:745-751

Moss SJ, Smart TG (2001) Constructing inhibitory synapses. Nat Rev Neurosci 2:240-250

Mulley JC, Nelson P, Guerrero S, Dibbens L, Iona X, McMahon JM, Harkin L, Schouten J, Yu S, Berkovic SF, Scheffer IE (2006) A new molecular mechanism for severe myoclonic epilepsy of infancy: exonic deletions in SCN1A. Neurology 67:1094-1095

Mulley JC, Scheffer IE, Harkin LA, Berkovic SF, Dibbens LM (2005) Susceptibility genes for complex epilepsy. Hum Mol Genet 14 Spec No. 2:R243-249

Nabbout R, Prud'homme JF, Herman A, Feingold J, Brice A, Dulac O, LeGuern E (2002) A locus for simple pure febrile seizures maps to chromosome 6q22-q24. Brain 125:2668-2680

Nadler JV, Perry BW, Cotman CW (1980) Selective reinnervation of hippocampal area CA1 and the fascia dentata after destruction of CA3-CA4 afferents with kainic acid. Brain Res 182:1-9

Nehrke K, Arreola J, Nguyen HV, Pilato J, Richardson L, Okunade G, Baggs R, Shull GE, Melvin JE (2002) Loss of hyperpolarization-activated Cl(-) current in salivary acinar cells from Clcn2 knockout mice. J Biol Chem 277:23604-23611

Newton MR, Berkovic SF, Austin MC, Rowe CC, McKay WJ, Bladin PF (1995) SPECT in the localisation of extratemporal and temporal seizure foci. J Neurol Neurosurg Psychiatry 59:26-30

Nicoll RA, Tomita S, Bredt DS (2006) Auxiliary subunits assist AMPA-type glutamate receptors. Science 311:1253-1256

Noda M, Ikeda T, Kayano T, Suzuki H, Takeshima H, Kurasaki M, Takahashi H, Numa S (1986) Existence of distinct sodium channel messenger RNAs in rat brain. Nature 320:188-192

Noebels JL (2003) The biology of epilepsy genes. Annu Rev Neurosci 26:599-625

Noebels JL, Fariello RG, Jobe PC, Lasley SM, Marescaux C (1997) Genetic models of generalized epilepsy. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 457-465

Oberbauer AM, Grossman DI, Irion DN, Schaffer AL, Eggleston ML, Famula TR (2003) The genetics of epilepsy in the Belgian tervuren and sheepdog. J Hered 94:57-63

Ohmori I, Kahlig KM, Rhodes TH, Wang DW, George AL, Jr. (2006) Nonfunctional SCN1A is common in severe myoclonic epilepsy of infancy. Epilepsia 47:1636-1642

Olsson I, Steffenburg S, Gillberg C (1988) Epilepsy in autism and autisticlike conditions. A population-based study. Arch Neurol 45:666-668

Onofri F, Giovedi S, Kao HT, Valtorta F, Bongiorno Borbone L, De Camilli P, Greengard P, Benfenati F (2000) Specificity of the binding of synapsin I to Src homology 3 domains. J Biol Chem 275:29857-29867

Ottman R (1997) Family studies. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 177-183

Ottman R, Risch N, Hauser WA, Pedley TA, Lee JH, Barker-Cummings C, Lustenberger A, Nagle KJ, Lee KS, Scheuer ML, et al. (1995) Localization of a gene for partial epilepsy to chromosome 10q. Nat Genet 10:56-60

Pal DK, Evgrafov OV, Tabares P, Zhang F, Durner M, Greenberg DA (2003) BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. Am J Hum Genet 73:261-270

Panayiotopoulos CP (2002) Idiopathic childhood occipital epilepsies. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 203-227

Panayiotopoulos CP, Obeid T (1989) Juvenile myoclonic epilepsy: an autosomal recessive disease. Ann Neurol 25:440-443

Pedley TA (1997) EEG traits. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 185-196

Pedley TA, Scheuwe ML, Walczak TS (1995) Epilepsy. In: Rowland LP (ed) Merritt's textbook of neurology. Williams & Wilkins, New York, pp 845-870

Pendfield W, Jasper H (1954) Epilepsy and the functional anatomy of the human brain. Churchill, London

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR (2002) GABA(A) receptor changes in delta subunit-deficient mice: altered expression of alpha4 and gamma2 subunits in the forebrain. J Comp Neurol 446:179-197

Phillips HA, Scheffer IE, Crossland KM, Bhatia KP, Fish DR, Marsden CD, Howell SJ, Stephenson JB, Tolmie J, Plazzi G, Eeg-Olofsson O, Singh R, Lopes-Cendes I, Andermann E, Andermann F, Berkovic SF, Mulley JC (1998) Autosomal dominant nocturnal frontal-lobe epilepsy: genetic heterogeneity and evidence for a second locus at 15q24. Am J Hum Genet 63:1108-1116

Picard F, Baulac S, Kahane P, Hirsch E, Sebastianelli R, Thomas P, Vigevano F, Genton P, Guerrini R, Gericke CA, An I, Rudolf G, Herman A, Brice A, Marescaux C, LeGuern E (2000) Dominant partial epilepsies. A clinical, electrophysiological and genetic study of 19 European families. Brain 123:1247-1262

Pieribone VA, Shupliakov O, Brodin L, Hilfiker-Rothenfluh S, Czernik AJ, Greengard P (1995) Distinct pools of synaptic vesicles in neurotransmitter release. Nature 375:493-497

Pinto D, Westland B, de Haan GJ, Rudolf G, da Silva BM, Hirsch E, Lindhout D, Trenite DG, Koeleman BP (2005) Genome-wide linkage scan of epilepsy-related photoparoxysmal electroencephalographic response: evidence for linkage on chromosomes 7q32 and 16p13. Hum Mol Genet 14:171-178

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815-850

Piven J, Palmer P, Landa R, Santangelo S, Jacobi D, Childress D (1997) Personality and language characteristics in parents from multiple-incidence autism families. Am J Med Genet 74:398-411

Plouin P, Anderson VE (2002) Benign neonatal convulsions familial or not. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 3-14

Porton B, Kao HT, Greengard P (1999) Characterization of transcripts from the synapsin III gene locus. J Neurochem 73:2266-2271

Puranam RS, Jain S, Kleindienst AM, Saxena S, Kim MK, Kelly Changizi B, Padma MV, Andrews I, Elston RC, Tiwari HK, McNamara JO (2005) A locus for generalized tonic-clonic seizure susceptibility maps to chromosome 10q25-q26. Ann Neurol 58:449-458

Ramus F (2001) Dyslexia. Talk of two theories. Nature 412:393-395

Rapin I (1997) Autism. N Engl J Med 337:97-104

Raskind WH, Igo RP, Chapman NH, Berninger VW, Thomson JB, Matsushita M, Brkanac Z, Holzman T, Brown M, Wijsman EM (2005) A genome scan in multigenerational families with dyslexia: Identification of a novel locus on chromosome 2q that contributes to phonological decoding efficiency. Mol Psychiatry 10:699-711

Rett AR, Teubel R (1964) Neugeborenenkrampfe im Rhamen einer epileptisch belasten Familie. Wien Klein Wschr 76:609-613

Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL, Jr. (2004) Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. Proc Natl Acad Sci U S A 101:11147-11152

Rich SS, Annegers JF, Hauser WA, Anderson VE (1987) Complex segregation analysis of febrile convulsions. Am J Hum Genet 41:249-257

Risch N (1990) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222-228

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251-255

Roger J, Bureau M, Ferrer-Vidal LO, Daurella LO, Saltarelli A. Genton P (1994) Clinical and electroencephalographic characteristics of idiopathic generalized epilepsies. In: Malafosse A, Genton P, Hirsch E, Marescaux C, Broglin D, Bernasconi R (eds) Idiopathic generalized epilepsies. John Libbey, London, pp 7-18

Rojas DC, Camou SL, Reite ML, Rogers SJ (2005) Planum temporale volume in children and adolescents with autism. J Autism Dev Disord 35:479-486

Rosahl TW, Geppert M, Spillane D, Herz J, Hammer RE, Malenka RC, Sudhof TC (1993) Short-term synaptic plasticity is altered in mice lacking synapsin I. Cell 75:661-670

Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Sudhof TC (1995) Essential functions of synapsins I and II in synaptic vesicle regulation. Nature 375:488-493

Rudy B (1978) Slow inactivation of the sodium conductance in squid giant axons. Pronase resistance. J Physiol 283:1-21

Sadamatsu M, Masui A, Sakai T, Kunugi H, Nanko S, Kato N (1999) Familial paroxysmal kinesigenic choreoathetosis: an electrophysiologic and genotypic analysis. Epilepsia 40:942-949

Sagane K, Hayakawa K, Kai J, Hirohashi T, Takahashi E, Miyamoto N, Ino M, Oki T, Yamazaki K, Nagasu T (2005) Ataxia and peripheral nerve hypomyelination in ADAM22-deficient mice. BMC Neurosci 6:33

Sander T, Schulz H, Saar K, Gennaro E, Riggio MC, Bianchi A, Zara F, et al. (2000) Genome search for susceptibility loci of common idiopathic generalised epilepsies. Hum Mol Genet 9:1465-1472

Schachter SC, Galaburda AM, Ransil BJ (1993) Associations of dyslexia with epilepsy, handedness, and gender. Ann N Y Acad Sci 682:402-403

Scheel H, Tomiuk S, Hofmann K (2002) A common protein interaction domain links two recently identified epilepsy genes. Hum Mol Genet 11:1757-1762

Scheffer IE, Berkovic SF (1997) Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. Brain 120:479-490

Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann E, Andermann F, Desbiens R, Keene D, Cendes F, et al. (1995) Autosomal dominant nocturnal frontal lobe epilepsy. A distinctive clinical disorder. Brain 118:61-73

Schiottz-Christensen E (1972) Genetic factors in febrile convulsions. An investigation of 64 same-sexed twin pairs. Acta Neurol Scand 48:538-546

Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, Kluck N, Manthey M, Plume E, Warnke A, Remschmidt H, Hulsmann J, Cichon S, Lindgren CM, Propping P, Zucchelli M, Ziegler A, Peyrard-Janvid M, Schulte-Korne G, Nothen MM, Kere J (2006) Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. Am J Hum Genet 78:52-62

Scott RC, King MD, Gadian DG, Neville BG, Connelly A (2003) Hippocampal abnormalities after prolonged febrile convulsion: a longitudinal MRI study. Brain 126:2551-2557

Senechal KR, Thaller C, Noebels JL (2005) ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. Hum Mol Genet 14:1613-1620

Shaywitz SE (1998) Dyslexia. N Engl J Med 338:307-312

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, Adamiker D (1999) Structure and subunit composition of GABA(A) receptors. Neurochem Int 34:379-385

Sik A, Smith RL, Freund TF (2000) Distribution of chloride channel-2-immunoreactive neuronal and astrocytic processes in the hippocampus. Neuroscience 101:51-65

Sillanpaa M, Koskenvuo M, Romanov K, Kaprio J (1991) Genetic factors in epileptic seizures: evidence from a large twin population. Acta Neurol Scand 84:523-526

Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M (1998) A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat Genet 18:25-29

Sirerol-Piquer MS, Ayerdi-Izquierdo A, Morante-Redolat JM, Herranz-Perez V, Favell K, Barker PA, Perez-Tur J (2006) The epilepsy gene LGI1 encodes a secreted glycoprotein that binds to the cell surface. Hum Mol Genet 15:3436-3445

Skradski SL, Clark AM, Jiang H, White HS, Fu YH, Ptacek LJ (2001) A novel gene causing a mendelian audiogenic mouse epilepsy. Neuron 31:537-544

Sloviter RS (1991) Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. Hippocampus 1:41-66

Sloviter RS (1994) The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. Ann Neurol 35:640-654

Smith MC (1997) Landau-Kleffner syndrome and continuous spikes and waves during slow sleep. In: Engel Jr J, Pedley TA (eds) Epilepsy: A Comprehensive textbook. Lippincott-Raven, New York, pp 2367-2377

Smith RD, Goldin AL (1998) Functional analysis of the rat I sodium channel in xenopus oocytes. J Neurosci 18:811-820

Snead OC, 3rd (1995) Basic mechanisms of generalized absence seizures. Ann Neurol 37:146-157

Spampanato J, Escayg A, Meisler MH, Goldin AL (2001) Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. J Neurosci 21:7481-7490

Spampanato J, Escayg A, Meisler MH, Goldin AL (2003) Generalized epilepsy with febrile seizures plus type 2 mutation W1204R alters voltage-dependent gating of Na(v)1.1 sodium channels. Neuroscience 116:37-48

Spampanato J, Kearney JA, de Haan G, McEwen DP, Escayg A, Aradi I, MacDonald BT, Levin SI, Soltesz I, Benna P, Montalenti E, Isom LL, Goldin AL, Meisler MH (2004) A novel epilepsy mutation in the sodium channel SCN1A identifies a cytoplasmic domain for beta subunit interaction. J Neurosci 24:10022-10034

Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF (1995) A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 11:201-203

Steriade M, Gloor P, Llinas RR, Lopes de Silva FH, Mesulam MM (1990) Report of IFCN Committee on Basic Mechanisms. Basic mechanisms of cerebral rhythmic activities. Electroencephalogr Clin Neurophysiol 76:481-508

Steriade M, McCormick DA, Sejnowski TJ (1993) Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679-685

Stuart G, Sakmann B (1995) Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. Neuron 15:1065-1076

Stuart GJ, Hausser M (2001) Dendritic coincidence detection of EPSPs and action potentials. Nat Neurosci 4:63-71

Sudhof TC, Czernik AJ, Kao HT, Takei K, Johnston PA, Horiuchi A, Kanazir SD, Wagner MA, Perin MS, De Camilli P, et al. (1989) Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins. Science 245:1474-1480

Sugawara T, Mazaki-Miyazaki E, Fukushima K, Shimomura J, Fujiwara T, Hamano S, Inoue Y, Yamakawa K (2002) Frequent mutations of SCN1A in severe myoclonic epilepsy in infancy. Neurology 58:1122-1124

Sugawara T, Mazaki-Miyazaki E, Ito M, Nagafuji H, Fukuma G, Mitsudome A, Wada K, Kaneko S, Hirose S, Yamakawa K (2001a) Nav1.1 mutations cause febrile seizures associated with afebrile partial seizures. Neurology 57:703-705

Sugawara T, Tsurubuchi Y, Agarwala KL, Ito M, Fukuma G, Mazaki-Miyazaki E, Nagafuji H, Noda M, Imoto K, Wada K, Mitsudome A, Kaneko S, Montal M, Nagata K, Hirose S, Yamakawa K (2001b) A missense mutation of the Na+ channel alpha II subunit gene Na(v)1.2 in a patient with febrile and afebrile seizures causes channel dysfunction. Proc Natl Acad Sci U S A 98:6384-6389

Sutula T, He XX, Cavazos J, Scott G (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science 239:1147-1150

Suzuki T, Delgado-Escueta AV, Aguan K, Alonso ME, Shi J, Hara Y, Nishida M, et al. (2004) Mutations in EFHC1 cause juvenile myoclonic epilepsy. Nat Genet 36:842-849

Szepetowski P, Rochette J, Berquin P, Piussan C, Lathrop GM, Monaco AP (1997) Familial infantile convulsions and paroxysmal choreoathetosis: a new neurological syndrome linked to the pericentromeric region of human chromosome 16. Am J Hum Genet 61:889-898

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, Lyytinen H, Muller K, Kaaranen M, Lindsberg PJ, Hannula-Jouppi K, Kere J (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci U S A 100:11553-11558

Tauer U, Lorenz S, Lenzen KP, Heils A, Muhle H, Gresch M, Neubauer BA, Waltz S, Rudolf G, Mattheisen M, Strauch K, Nurnberg P, Schmitz B, Stephani U, Sander T (2005) Genetic dissection of photosensitivity and its relation to idiopathic generalized epilepsy. Ann Neurol 57:866-873

Temkin O (1971) The falling sickness. A history of epilepsy from the Greeks to the beginnings of modern neurology. John Hopkins Press, Baltimore

Terada S, Tsujimoto T, Takei Y, Takahashi T, Hirokawa N (1999) Impairment of inhibitory synaptic transmission in mice lacking synapsin I. J Cell Biol 145:1039-1048

Thomas P, Genton P, Gelisse P, Wolf P (2002) Juvenile myoclonic epilepsy. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 335-355

Trescher WH, Lesser RP (1996) The Epilepsies. In: Bradley WG, Daroff RB, Fenichel GM, Marsden CD (eds) Neurology in clinical practice. Newton: Butterworth-Heinemann, pp 1625-1654

Trimble MR (1987) Anticonvulsant drugs and cognitive function: a review of the literature. Epilepsia 28 Suppl 3:S37-45

Tu Y, Nayak SK, Woodson J, Ross EM (2003) Phosphorylation-regulated inhibition of the Gz GTPase-activating protein activity of RGS proteins by synapsin I. J Biol Chem 278:52273-52281

Turnbull J, Lohi H, Kearney JA, Rouleau GA, Delgado-Escueta AV, Meisler MH, Cossette P, Minassian BA (2005) Sacred disease secrets revealed: the genetics of human epilepsy. Hum Mol Genet 14:2491-2500

Turski L, Niemann W, Stephens DN (1990) Differential effects of antiepileptic drugs and beta-carbolines on seizures induced by excitatory amino acids. Neuroscience 39:799-807

Vadlamudi L, Andermann E, Lombroso CT, Schachter SC, Milne RL, Hopper JL, Andermann F, Berkovic SF (2004) Epilepsy in twins: insights from unique historical data of William Lennox. Neurology 62:1127-1133

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, et al. (2001) The sequence of the human genome. Science 291:1304-1351

Vitko I, Chen Y, Arias JM, Shen Y, Wu XR, Perez-Reyes E (2005) Functional characterization and neuronal modeling of the effects of childhood absence epilepsy variants of CACNA1H, a T-type calcium channel. J Neurosci 25:4844-4855

von Krosigk M, Bal T, McCormick DA (1993) Cellular mechanisms of a synchronized oscillation in the thalamus. Science 261:361-364

Vreugdenhil M, Hoogland G, van Veelen CW, Wadman WJ (2004) Persistent sodium current in subicular neurons isolated from patients with temporal lobe epilepsy. Eur J Neurosci 19:2769-2778

Wallace RH, Berkovic SF, Howell RA, Sutherland GR, Mulley JC (1996) Suggestion of a major gene for familial febrile convulsions mapping to 8q13-21. J Med Genet 33:308-312

Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF (2001a) Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49-52

Wallace RH, Scheffer IE, Barnett S, Richards M, Dibbens L, Desai RR, Lerman-Sagie T, Lev D, Mazarib A, Brand N, Ben-Zeev B, Goikhman I, Singh R, Kremmidiotis G, Gardner A, Sutherland GR, George AL, Jr., Mulley JC, Berkovic SF (2001b) Neuronal sodium-channel alpha1-subunit mutations in generalized epilepsy with febrile seizures plus. Am J Hum Genet 68:859-865

Wallace RH, Wang DW, Singh R, Scheffer IE, George AL, Jr., Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC (1998) Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat Genet 19:366-370

Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, Becker LE, MacDonald JF, Wang YT (1997) Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. Nature 388:686-690

Weissbecker KA, Durner M, Janz D, Scaramelli A, Sparkes RS, Spence MA (1991) Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. Am J Med Genet 38:32-36

Westenbroek RE, Merrick DK, Catterall WA (1989) Differential subcellular localization of the RI and RII Na+ channel subtypes in central neurons. Neuron 3:695-704

Wilson JV, Reynolds EH (1990) Texts and documents. Translation and analysis of a cuneiform text forming part of a Babylonian treatise on epilepsy. Med Hist 34:185-198

Winawer MR, Ottman R, Hauser WA, Pedley TA (2000) Autosomal dominant partial epilepsy with auditory features: defining the phenotype. Neurology 54:2173-2176

Wolf P (1992) Juvenile myoclonic epilepsy. In : Roger J, Bureau M, Dravet C, Dreifuss FE, Perret A, Wolf P (eds) Epileptic Syndromes in Infancy and Adolescence (2^{nd} edn). John Libbey, London, pp 313-327

Wolf P (2002) Epilepsy with grand mal on awakening. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 357-368

Wolf P (2003) Of cabbages and kings: some considerations on classifications, diagnostic schemes, semiology, and concepts. Epilepsia 44:1-13

Wolf P, Inoue Y (2002) Juvenile absence epilepsy. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P (eds) Epileptic syndromes in infancy, childood and adolescence. John Libbey, London, pp 331-334

Xiao W, Oefner PJ (2001) Denaturing high-performance liquid chromatography: A review. Hum Mutat 17:439-474

Xiong L, Labuda M, Li DS, Hudson TJ, Desbiens R, Patry G, Verret S, Langevin P, Mercho S, Seni MH, Scheffer I, Dubeau F, Berkovic SF, Andermann F, Andermann E, Pandolfo M (1999) Mapping of a gene determining familial partial epilepsy with variable foci to chromosome 22q11-q12. Am J Hum Genet 65:1698-1710

Yeh GC, Bonhaus DW, Nadler JV, McNamara JO (1989) N-methyl-D-aspartate receptor plasticity in kindling: quantitative and qualitative alterations in the N-methyl-D-aspartate receptor-channel complex. Proc Natl Acad Sci U S A 86:8157-8160

Zara F, Bianchi A, Avanzini G, Di Donato S, Castellotti B, Patel PI, Pandolfo M (1995) Mapping of genes predisposing to idiopathic generalized epilepsy. Hum Mol Genet 4:1201-1207

Zuberi SM, Eunson LH, Spauschus A, De Silva R, Tolmie J, Wood NW, McWilliam RC, Stephenson JB, Kullmann DM, Hanna MG (1999) A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. Brain 122:817-825

APPENDIX 1: MOLECULAR ANALYSIS OF THE A322D MUTATION IN THE $GABA_A$ RECEPTOR α1-SUBUNIT CAUSING JUVENILE MYOCLONIC EPILEPSY

Reference :

Krampfl K, Maljevic S, Cossette P, Ziegler E, Rouleau GA, Lerche H, and Bufler J. Molecular analysis of the A322D mutation in the $GABA_A$ receptor α1-subunit causing juvenile myoclonic epilepsy. *European Journal of Neuroscience* 2005; 22: 10-20.

European Journal of Neuroscience, Vol. 22, pp. 10–20, 2005

Molecular analysis of the A322D mutation in the $GABA_A$ receptor α_1-subunit causing juvenile myoclonic epilepsy

Klaus Krampfl,[1,2] Snezana Maljevic,[3] Patrick Cossette,[4] Elke Ziegler,[1,2] Guy A. Rouleau,[4] Holger Lerche[3] and Johannes Bufler[1,2]
[1]Neurologische Klinik, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Center for Systems Neuroscience (ZSN), Hannover, Germany
[3]Neurologische Klinik und Abteilung für Angewandte Physiologie, Universität Ulm, Ulm, Germany
[4]Service de Neurologie et Centre de Recherche, Centre Hospitalier de l'Université de Montréal – Hôpital Notre-Dame, Montréal, PQ, Canada

Keywords: fast application, fluorescence imaging, idiopathic epilepsy, ligand-gated ion channel, recombinant receptor

Abstract

Juvenile myoclonic epilepsy (JME) belongs to the most common forms of hereditary epilepsy, the idiopathic generalized epilepsies. Although the mode of inheritance is usually complex, mutations in single genes have been shown to cause the disease in some families with autosomal dominant inheritance. The first mutation in a multigeneration JME family has been recently found in the α_1-subunit of the $GABA_A$ receptor (*GABRA1*), predicting the single amino acid substitution A322D. We further characterized the functional consequences of this mutation by coexpressing α_1-, β_2- and γ_2-subunits in human embryonic kidney (HEK293) cells. By using an ultrafast application system, mutant receptors have shown reduced macroscopic current amplitudes at saturating GABA concentrations and a highly reduced affinity to GABA compared to the wild-type (WT). Dose–response curves for current amplitudes, activation kinetics, and GABA-dependent desensitization parameters showed a parallel shift towards 30- to 40-fold higher GABA concentrations. Both deactivation and resensitization kinetics were considerably accelerated in mutant channels. In addition, mutant receptors labelled with enhanced green fluorescent protein (EGFP) were not integrated in the cell membrane, in contrast to WT receptors. Therefore, the A322D mutation leads to a severe loss-of-function of the human $GABA_A$ receptor by several mechanisms, including reduced surface expression, reduced GABA-sensitivity, and accelerated deactivation. These molecular defects could decrease and shorten the resulting inhibitory postsynaptic currents (IPSCs) *in vivo*, which can induce a hyperexcitability of the postsynaptic membrane and explain the occurrence of epileptic seizures.

Introduction

Epileptic seizures are caused by abnormal, paroxysmal, synchronized discharges of neurons in the brain that can be induced by structural lesions such as tumours or stroke, or by genetic alterations. The idiopathic generalized epilepsies (IGE) are the most common inherited forms of epilepsy, comprising four major subtypes: childhood and juvenile absence epilepsy, juvenile myoclonic epilepsy (JME) and epilepsy with Grand-Mal seizures on awakening. While the mode of inheritance of IGE overall is rather complex, an increasing number of families with Mendelian inheritance have been identified, suggesting a single genetic defect with high penetrance. Due to the fundamental role of ion channels in membrane excitability, these proteins were always suspected to participate in the pathogenesis of epilepsy, but it is only recently, that direct evidence proving this assumption has been provided. Indeed, in the recent ten years, mutations in ion channel encoding genes have been detected as an underlying cause of different idiopathic epileptic syndromes with autosomal dominant inheritance (Mulley *et al.*, 2003; Noebels, 2003; Lerche *et al.*, 2004). Specifically, mutations in the γ_2-subunit of the $GABA_A$ receptors have been described in generalized epilepsy with febrile seizures plus ($GEFS^+$) and related syndromes, in which afebrile and febrile seizures occur very heterogeneously in large pedigrees (Baulac *et al.*, 2001; Wallace *et al.*, 2001; Harkin *et al.*, 2002; Kananura *et al.*, 2002). In families with classical forms of IGE without febrile seizures, the first mutation has been identified in a family with JME in the α_1-subunit of the $GABA_A$ receptor, in which an alanine in the third transmembrane domain is replaced by an aspartate (A322D) (Cossette *et al.*, 2002). Mutations in the chloride channel ClC-2, a protein involved in maintaining the transmembrane chloride gradient essential for an inhibitory GABA response, were recently detected in all four common suptypes of IGE (Haug *et al.*, 2003). Thus, impaired GABAergic synaptic inhibition seems to be a common pathophysiological mechanism in generalized idiopathic epilepsy syndromes ($GEFS^+$ and IGE).

$GABA_A$ receptor channels mediate fast inhibitory synaptic transmission in the brain. Upon binding of GABA, the channels open a chloride pore allowing the passive flow of chloride ions following their transmembrane gradient. When the intracellular chloride concentration is physiologically low, this leads to a stabilization of the membrane potential near the resting potential by membrane hyperpolarization or by 'shunting' depolarizing synaptic inputs. A great diversity of $GABA_A$ receptor subtypes with distinct kinetic and

Correspondence: Dr Klaus Krampfl, [1]Neurologische Klinik, or Dr Holger Lerche, [3]Neurologische Klinik, as above.
E-mail: krampfl.klaus@mh-hannover.de or holger.lerche@medizin.uni-ulm.de

Received 7 January 2005, revised 8 April 2005, accepted 18 April 2005

doi:10.1111/j.1460-9568.2005.04168.x

pharmacological properties is generated by different combinations of subunits. Based on their sequence homologies, the cloned subunits are grouped into six families (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, π). Functional channels occur as pentameric heteromers. The most common subunit combination in the brain is two α_1-, two β_2- and one γ_2-subunit (Mohler *et al.*; 1996; Mehta & Ticku, 1999; Sieghart & Sperk, 2002).

The A322D mutation of the α_1-subunit causing juvenile myoclonic epilepsy has been shown to reduce the maximal peak current amplitude of macroscopic GABAergic currents by shifting the dose–response curve for GABA to much higher concentrations (Cossette *et al.*, 2002). Loss-of-function mechanisms have been also described for the other $GABA_A$ receptor mutations detected in the γ_2-subunit (Baulac *et al.*, 2001; Wallace *et al.*, 2001; Bianchi *et al.*, 2002; Ramakrishnan & Hess, 2004). A more detailed electrophysiological analysis of the A322D mutation, when inserted in rat $GABA_A$ receptor channels, additionally revealed a shorter deactivation phase of macroscopic currents as well as a shorter open dwell time of single channel openings (Fisher, 2004).

GABA-induced currents are characterized by a rapid rising phase in the submillisecond range, concentration-dependent triphasic desensitization kinetics and fast deactivation after cessation of the agonist application (Jones & Westbrook, 1995; Haas & Macdonald, 1999; Krampfl *et al.*, 2000; Schlesinger *et al.*, 2004). These kinetic details can be only resolved with an ultrafast perfusion technique mimicking release of GABA into the synaptic cleft and inducing the following postsynaptic receptor activation (Franke *et al.*, 1987). The main goal of our study therefore was to further characterize the functional impact of the A322D mutation causing autosomal dominant JME in humans. For this purpose, we applied the patch-clamp technique in combination with ultrafast agonist application to study the kinetic details of the A322D mutation. In addition, we studied surface expression of the mutant and wild-type (WT) receptors using tagged constructs linked to the green fluorescent protein in combination with confocal laser scanning microscopy.

Materials and methods

cDNA constructs

cDNAs encoding the three different human $GABA_A$ subunits (α_1, β_2 and γ_2) were previously subcloned into the pcDNA3.1(–) vector (Invitrogen Corporation, Carlsbad, CA), and the mutation A322D introduced using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA; Cossette *et al.*, 2002). For confocal fluorescence studies, the enhanced green fluorescent protein (EGFP) coding sequence from the pEGFP-C1 vector (Clontech, Palo Alto, CA) was inserted at the N-terminus, between amino acids four and five of the mature WT and mutated $GABA_A$ α_1-subunit using a PCR-based recombination technique. This position was shown to be functionally silent for the γ_2-subunit (Kittler *et al.*, 2000). All constructs were completely sequenced to confirm the mutation and insertion of EGFP.

Cell culture and transfection

Human embryonic kidney (HEK293) cells were cultured and transiently transfected by electroporation or calcium-phosphate using standard procedures as previously described (Krampfl *et al.*, 2002a). Transient cotransfections were performed in a ratio of 1 : 1 : 2 for $\alpha_1 : \beta_2 : \gamma_2$ or $\alpha_{1\text{-A322D}} : \beta_2 : \gamma_2$, and 1 : 1 : 1 : 2 for cotransfection of α_1, $\alpha_{1\text{-A322D}}$, β_2, and γ_2. Transfected cells were replated on glass coverslips in DMEM containing 10% fetal calf serum (FCS) and incubated for 15–18 h.

Electrophysiological recordings

Patch-clamp measurements for fast application experiments were performed on outside-out excised patches or small cells lifted from the bottom in the whole cell mode. Patch pipettes were pulled from borosilicate glass tubes in two stages with a horizontal DMZ pipette puller (Zeitz Instruments, Augsburg, Germany). They were coated with Sylgard and fire-polished. Pipette resistance was 4–8 MΩ for whole cell recordings and 7–10 MΩ for outside-out recordings when filled with intracellular solution containing (in mM): 140 KCl, 11 EGTA, 10 Hepes, 10 glucose, 2 $MgCl_2$. HEK293 cells were superfused with an extracellular solution containing (in mM): 162 NaCl, 5.3 KCl, 2 $CaCl_2$, 0.67 NaH_2PO_4, 0.22 KH_2PO_4, 15 Hepes, 5.6 glucose. The pH of both solutions was adjusted to 7.3. The osmolarity of both solutions was adjusted to 340 mosm/L with mannitol. Using this solutions with relatively high osmolarity in experiments with HEK293 cells, we observed stable recording conditions that did allow for whole cell experiments lasting longer than 30 min (Krampfl *et al.*, 2002a,b). There were no junction potentials and the extracellular and intracellular solutions used should not influence the analysed parameters of the recorded ionic currents through $GABA_A$ receptor channels that were investigated in our study. The holding potential was kept between −40 and −80 mV except for experiments on voltage dependence when the holding potential was varied between +60 and −80 mV (no rectification observed). Data were recorded with an Axopatch200B patch-clamp amplifier (Axon Instruments, Union City, CA, USA). For outside-out recordings, the low-pass filter of the Axopatch200B patch-clamp amplifier was set at 100 kHz, and ensemble currents were sampled with 20 kHz using a Digidata 1200 Interface and pCLAMP6 software (Axon Instruments, Union City, CA, USA). For further analysis, data were digitally filtered at 5 kHz. Whole cell recordings were performed with the low-pass filter set at 2 kHz and a sampling rate of 5 kHz. The ANOVA routine of ORIGIN 7 software (OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis. Differences were considered significant at the $P < 0.05$ or higher levels. All data were given as means ± SEM.

Fast application technique and data evaluation

A piezo-driven ultrafast perfusion system was used for application of the neurotransmitter GABA (0.1 μM–100 mM) to excised outside-out membrane patches or small cells, as previously described (Franke *et al.*, 1987; Krampfl *et al.*, 2002a,b). With our system, the time for solution exchange was regularly < 100 μs and at best ≈ 50 μs (10–90% rise time). It was estimated by measurements of liquid junction potentials with a ten-fold difference in ionic strength at the tip of leaky patch pipettes at the end of the experiments. GABA or phenobarbital was applied using agonist pulses of 1, 5, 400, or 2000 ms duration and with intervals ranging from 30 to 3000 ms between two successive 5 ms pulses in paired pulse experiments. For paired pulse inhibition analysis the current remaining from the first pulse (in case of brief interpulse intervals) was subtracted from the absolute peak of the second amplitude, thus generating a relative amplitude measurement (Bianchi *et al.*, 2002; Mohammadi *et al.*, 2003). Dose–response experiments were performed on small cells lifted from the bottom because of more stable recording conditions. Pulse protocols were designed with alternating applications of a saturating concentration and lower concentrations for normalization of the current responses to lower concentrations of agonist to that upon saturating agonist concentrations. Each parameter at a nonsaturating agonist concentration was normalized to the respective mean value

derived from current traces at saturating concentrations of agonist before and after trains of applications of lower concentrations. By this approach, a potential influence of current run-down on dose–response experiments was ruled out. For kinetic analysis in both whole cell and outside-out recordings, the Simplex algorithm of the pCLAMP6 software was applied to fit first to third order exponential functions to the data points. Further exponential components were introduced until they did not significantly improve the quality of the fit (Haas & Macdonald, 1999). Curve fitting procedure was started with a single exponential for each average current curve. Further exponentials were added until the fit software showed no further time constant of the respective curve. The standard deviation of each fit with was noted as given by the pCLAMP6 fit software (Mohammadi *et al.*, 2003). These standard deviation values were statistically tested for significance of difference of their means (ANOVA). In case of a significantly reduced standard deviation of the fit it was chosen. GABA and phenobarbital were obtained from SIGMA (Taufkirchen, Germany).

Immunoblot analysis

HEK293 cells were collected 48 h after transfection and lysed on ice in a buffer containing 50 mM Tris-HCl (pH 7.2), 150 mM NaCl, 0.5% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 2.1 μg/mL aprotinin, 2 μg/mL pepstatin and 1 mM phenylmethylsulphonyl fluoride. After 15 min of centrifugation at 10 000 × *g*, the protein concentration in the supernatant was measured using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and the same amount of total protein for each transfection group was loaded in SDS sample buffer, separated on a 8% SDS–PAGE gel and then transferred to a

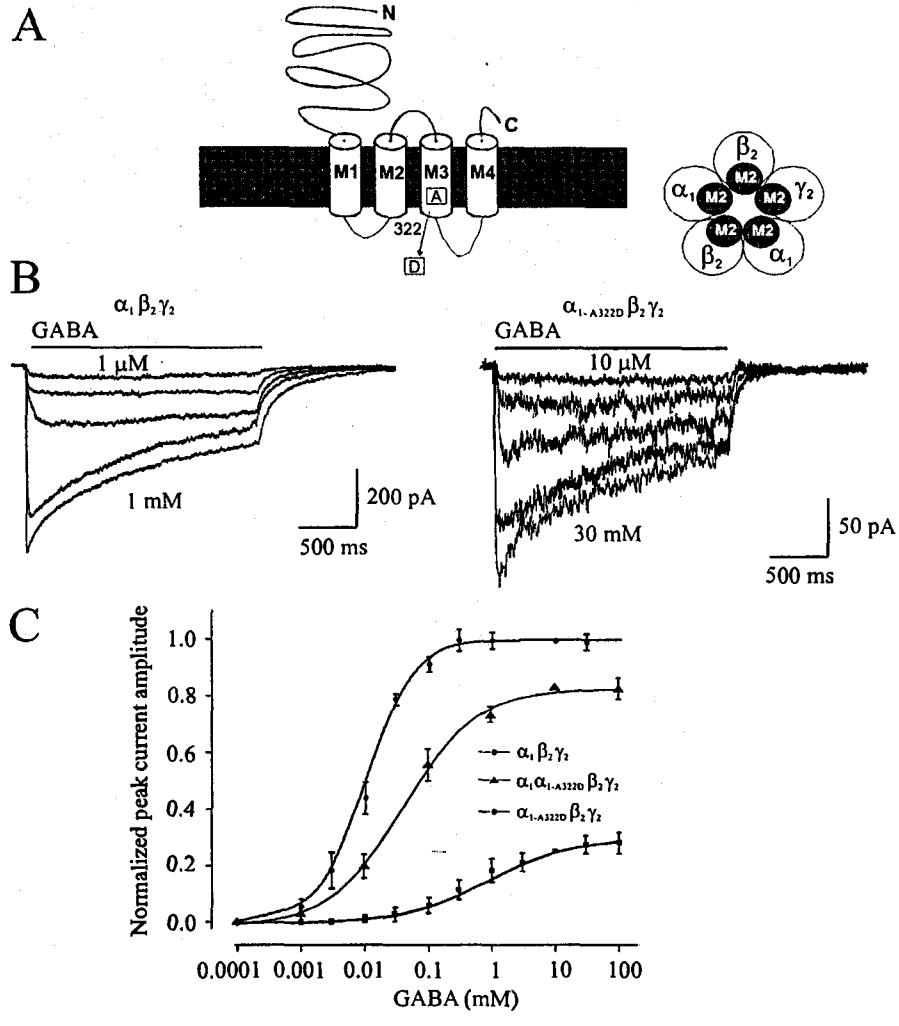


FIG. 1. (A) Schematic view of the point mutation A322D in the third transmembrane domain of the human α_1-subunit and the supposed pentameric structure of the $GABA_A$ receptor composed of two α_1-subunits, two β_2-subuits and one γ_2-subunit. (B) GABA concentration–response relationship for $\alpha_1\beta_2\gamma_2$ and $\alpha_{1\text{-}A322D}\beta_2\gamma_2$. Examples of individual whole cell current traces upon application of different concentrations of GABA as indicated. Different amplitude scales were used for WT ($\alpha_1\beta_2\gamma_2$) and mutant ($\alpha_{1\text{-}A322D}\beta_2\gamma_2$) receptors. The holding membrane potential was −60 mV. Fast application of GABA at different concentrations was carried out using a piezo-driven device. (C) Dose–response analysis. The peak current amplitude of individual GABA-induced currents from both WT (circles) and mutant receptors (squares) was normalized to the maximal response from the wild-type receptors and plotted against the concentration of GABA. The Hill equation was fitted to the data points, and the EC_{50} (concentration of a drug that produces 50% of its maximal effect, exact values are given in the text) and the Hill coefficient (n_H) were determined. EC_{50} values of GABA were 11.2 ± 0.6 μM for the WT (n_H = 1.35), and 485 ± 40 μM for mutant receptor channels (n_H = 0.80). The A322D mutation results in a lowered maximum response, and a rightward shift of the dose–response relation. In case of cotransfection of WT and mutant α_1-subunits an intermediate EC_{50} of GABA of 40.4 ± 7.7 μM (n = 5) with n_H = 0.92 was observed (triangles).

nitrocellulose membrane. The blot was probed with a polyclonal antibody against EGFP (1 : 500; Chemicon, Temecula, CA, USA) and with a horseradish peroxidase–conjugated goat antibody against rabbit IgG (1 : 2500), and developed using the ECL Western blot detection system (Amersham Biosciences, Piscataway, NJ, USA).

Confocal imaging

EGFP-tagged WT and mutant $GABA_A$ α_1-subunits were cotransfected with β_2- and γ_2-subunits into HEK293 cells. Twenty-four hours after transfection, cells were washed with PBS, treated with trypsin and plated on glass cover slips coated with Poly L-Lysine. Images were obtained using a confocal laser scanning microscope (Radiance, Bio-Rad Hercules, CA, USA) 24–48 h after transfection. EGFP was excited with the 488-nm line of the argon ion laser. For detection of fluorescence we used the band-pass filter 515/30 nm. A membrane-labelling dye, FM4-64 (Molecular Probes Inc., Eugene, OR, USA) was applied in a final concentration of 5 μM. The dsRed2-ER vector, a marker for the endoplasmic reticulum, was obtained from Clontech (Palo Alto, CA, USA) and transfected into HEK293 cells. The fluorescence of both markers was measured using a HeNe laser, excitation at 543 nm, emission at >570 nm. The laser power was adjusted individually to obtain final images of equivalent brightness. Therefore, differences in brightness do not reflect differences in efficiency of protein expression among the constructs. Images were processed using Adobe Photoshop software (ADOBE Systems, Mountain View, CA, USA).

Results

For electrophysiological recordings, wild-type (WT) or mutant (A322D) human $GABA_A$ receptor α_1-subunits (Fig. 1A) were cotransfected with β_2- and γ_2-subunits in a 1 : 1 : 2 ratio in HEK293 cells. The whole cell or outside-out patch clamp technique was applied in combination with a piezo-driven fast solution exchange. Dose–response relationships were determined for activation, desensitization, and deactivation of $GABA_A$ receptors in whole cell experiments due to the more stable recording conditions and larger currents as compared to outside-out patches. However, as it is known from previous studies with glutamate and $GABA_A$ receptor channels using fast solution exchange techniques (Partin *et al.*, 1996; Krampfl *et al.*, 2000, 2002a, 2002b), the kinetics of all GABA-dependent gating processes are estimated considerably slower in whole cell experiments compared to those using outside-out patches. Therefore, the gating kinetics were additionally determined for WT and mutant receptors using recordings in the outside-out configuration at saturating GABA concentrations.

Dose-dependent activation of WT and mutant $GABA_A$ receptors

GABA was applied in 2-s-lasting pulses to lifted cells every 30 s to avoid current run down or accumulation of receptor channels in long-lived desensitized states (Haas & Macdonald, 1999; Krampfl *et al.*, 2000). As can be observed in Fig. 1B, the peak current amplitude increased seven- to eight-fold when the GABA concentration was raised from 0.1 μM to 30 mM, and the current rise time decreased. For the construction of dose–response curves, which are shown in Fig. 1C as semilogarithmic plots, the peak current amplitude was normalized to the one obtained after application of 10 mM GABA. The curve was fitted using the Hill equation with a Hill coefficient (n_H) of 1.35 and 0.80 for WT and mutant channels. The EC_{50} was determined to 11.2 ± 0.6 μM GABA for the WT, and 485 ± 40 μM for mutant ($\alpha_{1\text{-}A322D}\beta_2\gamma_2$) $GABA_A$ receptor channels ($P = 0.0004$), showing an approximately 40-fold increase of the EC_{50} for the mutant (Fig. 1C). To mimic the *in vivo* situation in affected, heterozygous patients, equal amounts of mutant and WT α_1-subunits were cotransfected together with β_2 and γ_2 in a 1 : 1 : 1 : 2 ratio. We obtained an intermediate EC_{50} of 40.4 ± 7.7 μM GABA ($n_H = 0.92$) for these experiments ($n = 5$, Fig. 1C). It was significantly different from the EC_{50} at WT receptors ($P = 0.018$) as well as mutant receptors ($P = 0.0006$).

Saturation was reached at 1 mM GABA for the WT, similar to previous studies of our group (Krampfl *et al.*, 2000), and at 30 mM GABA for mutant receptors. The mean peak current amplitude, as determined at saturating GABA concentrations in whole cell measurements using small cells, was reduced by approximately four-fold for mutant receptors; 624 ± 180 pA ($n = 16$) and 161 ± 27 pA ($n = 18$) for WT and A322D, respectively ($P = 0.0006$). As the single channel conductance was similar for both receptor types (30 ± 3 pS for WT, $n = 970$ events in three different patches, and 29 ± 4 pS for A322D mutant channels, $n = 1211$ events in four different patches), and as there was no difference in voltage dependence (data not shown), these results also suggest a considerably reduced surface expression of A322D mutant receptors.

Rise time of GABA-induced currents

The activation kinetics of currents recorded from ligand-gated ion channels upon fast agonist application are closely related to agonist binding (Franke *et al.*, 1993; Clements *et al.*, 1998; Ratner *et al.*, 2000; Krampfl *et al.*, 2002b). In addition to the observed rightward shift of the peak current dose–response curve of mutant compared to WT receptors, a corresponding behaviour of activation kinetics would therefore support an underlying pathomechanism related to agonist binding affinity or efficacy. As expected, we observed a dose-dependent decrease of the current rise time for WT and mutant receptor channels upon application of increasing GABA concentrations. The double logarithmic plot of Fig. 2 (data from whole cell measurements) reveals a large parallel rightward shift of the current rise time dose–response curve for A322D mutant compared to WT receptors, similar to that observed for current amplitudes (Fig. 1C).

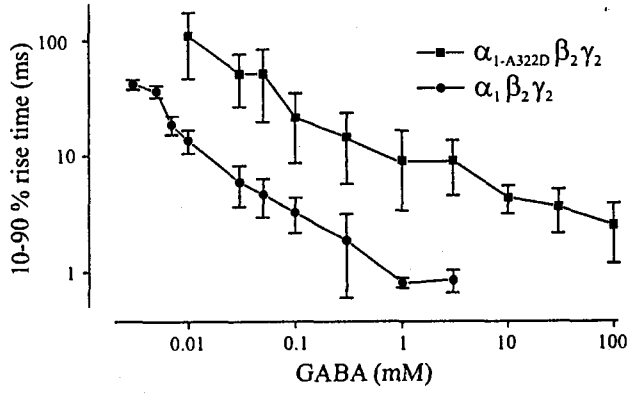


FIG. 2. Analysis of current rise times in whole cell recordings. Dose dependence of the 10–90% current rise time in whole cell experiments shown as a semilogarithmic plot as in Fig. 1C. A rightward shift of the dose–response curve towards higher GABA concentrations is observed for mutant channels.

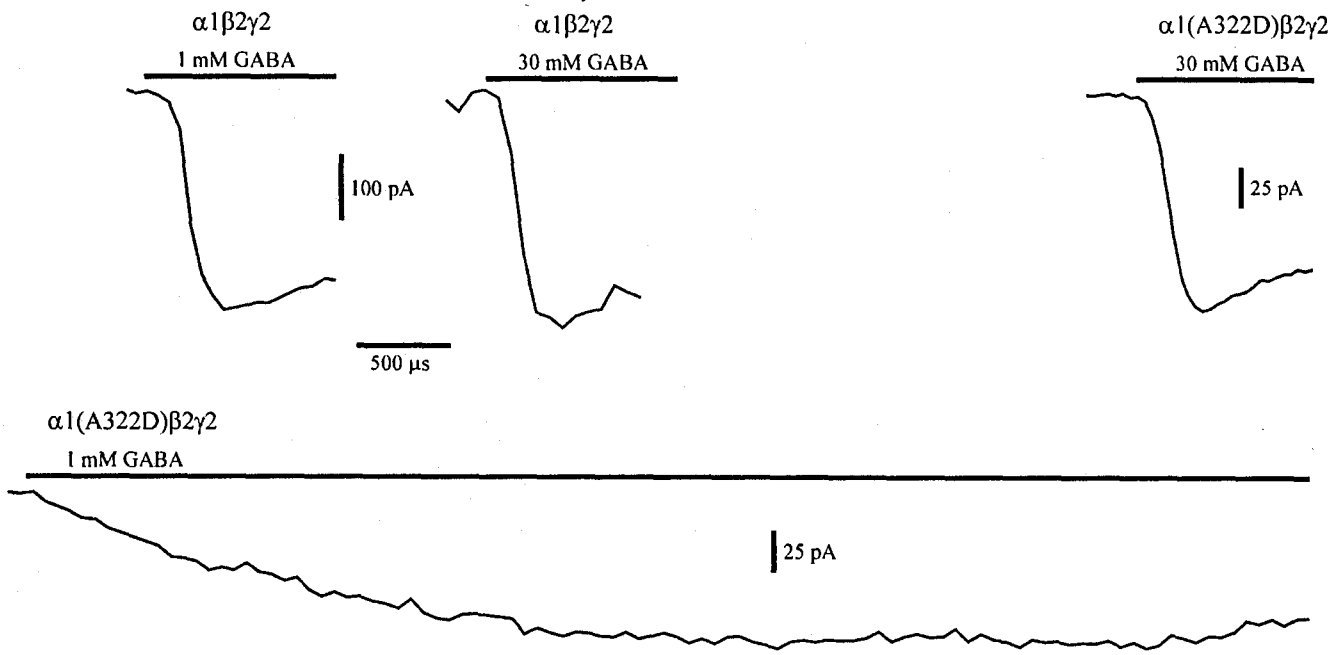


FIG. 3. Examples of the current rising phase recorded from outside-out patches upon ultrafast application of high agonist concentrations. In the experiments shown, the 10–90% current rise time was 167 µs and 153 µs when 1 mM or 30 mM GABA was applied to WT (upper left and middle current trace), and 156 µs when 30 mM GABA was applied to mutant receptors (upper right current trace). Upon fast application of 1 mM GABA to mutant receptors, the current developed with a 10–90% rise time of 1890 µs (lower current trace).

As a fast agonist application in the µs range is required for the exact kinetic analysis of the current rising phase at high agonist concentrations, we conducted those experiments also with outside-out patches. To compare the kinetics of WT and mutant receptors, 1 mM for WT and 1 or 30 mM GABA for mutant receptor channels were applied. Raw currents recorded from outside-out patches showing examples of the rising phase are presented in Fig. 3. The 10–90% rise times upon application of 1 mM for WT and 1 or 30 mM GABA for mutant receptors are given in Table 1. Upon application of 1 mM GABA, the rise time for mutant receptors was prolonged approximately six-fold compared to the WT ($P = 2.2 \times 10^{-15}$), but with 30 mM GABA, the values for the mutant were similar to those obtained with 1 mM GABA for the WT (no significant difference, $P = 0.11$). These data reflect the 30- to 40-fold difference in GABA sensitivity but do not indicate a fundamental difference in the mechanism of channel gating (Fig. 3).

Dose dependence and kinetics of desensitization

Desensitization of inhibitory ionic receptors, i.e. a current decay in the presence of the agonist, is one of the parameters that determines

TABLE 1. Kinetic analysis of WT and mutant A322D $GABA_A$ receptors using outside-out patches and ultrafast agonist application

	WT, 1 mM GABA	A322D, 1 mM GABA	A322D, 30 mM GABA
Rise time (10–90%, µs)	371 ± 32 ($n = 28$)	2060 ± 120 ($n = 4$)	447 ± 31 ($n = 43$)
Desensitization	$n = 20$	$n = 10$	$n = 25$
$\tau_{des\text{-}fast}$ (ms)	8.5 ± 1.2	16.6 ± 7.1	8.1 ± 1.0
$\tau_{des\text{-}int}$ (ms)	105.1 ± 8.0	75.4 ± 9.1	89.1 ± 14.0
$\tau_{des\text{-}slow}$ (ms)	866 ± 79	431.3 ± 55.0	550 ± 70
$a_{des\text{-}fast}$	0.26 ± 0.03	0.08 ± 0.03	0.34 ± 0.03
$a_{des\text{-}int}$	0.33 ± 0.09	0.29 ± 0.04	0.27 ± 0.02
$a_{des\text{-}slow}$	0.41 ± 0.03	0.63 ± 0.02	0.39 ± 0.03
c_{des}	0.10 ± 0.04	0.14 ± 0.04	0.11 ± 0.02
Deactivation	$n = 10$	–	$n = 15$
$\tau_{deact\text{-}fast}$ (ms)	20.8 ± 2.4	–	7.6 ± 1.1
$\tau_{deact\text{-}slow}$ (ms)	212 ± 41	–	89 ± 28
$a_{deact\text{-}fast}$	0.57 ± 0.04	–	0.66 ± 0.04
Resensitization	$n = 11$	–	$n = 13$
$\tau_{res\text{-}fast}$ (ms)	64	–	45
$\tau_{res\text{-}slow}$ (ms)	544	–	–
$a_{res\text{-}fast}$	0.49	–	–

'τ' stands for the respective time constants of desensitization, deactivation, and resensitization; '*a*' for the relative amplitudes of the respective time constants and '*c*' for the steady state current amplitude normalized to the peak current amplitude. '*c*' was determined independently from the time constants, that is why the sum of the relative amplitudes '*a*' is equal to 1.

the time course of inhibitory postsynaptic currents (IPSCs). Its dose dependence can be determined in concentration jump experiments in which the agonist concentration can be exactly controlled during prolonged applications. Currents in whole cell recordings were nondesensitizing for up to 1 μM GABA for WT or 10 μM for mutant A322D receptors. With higher GABA concentrations, desensitization was increasingly accelerated. Its time course was quantified by fitting first to third order exponential functions to the current decay. The desensitization time constants were denoted as $\tau_{des\text{-}fast}$, $\tau_{des\text{-}int}$ and $\tau_{des\text{-}slow}$ for the fastest, intermediate and slowest components, respectively (Fig. 4A and B). With increasing GABA concentrations, the time constants of desensitization tended to decrease only slightly (Fig. 4A and B), while faster components were continuously added and their relative contribution increased steadily (Fig. 4A–D). The concentration dependence for the WT was very similar as previously described for rodent receptors (Haas & Macdonald, 1999; Krampfl *et al.*, 2000), whereas for mutant receptors it was shifted towards 30- to 40-fold higher concentrations (Figs 1B, and 4A and B).

Desensitization time constants and their relative amplitudes as obtained from GABA-induced currents recorded in outside-out patches are presented in Table 1. The main difference between WT and mutant receptors at 1 mM GABA was a distinct decrease of the relative amplitude of the fast component, which was only detectable in one-third of the recordings with mutant receptors but in all recordings derived from the WT. There was no significant

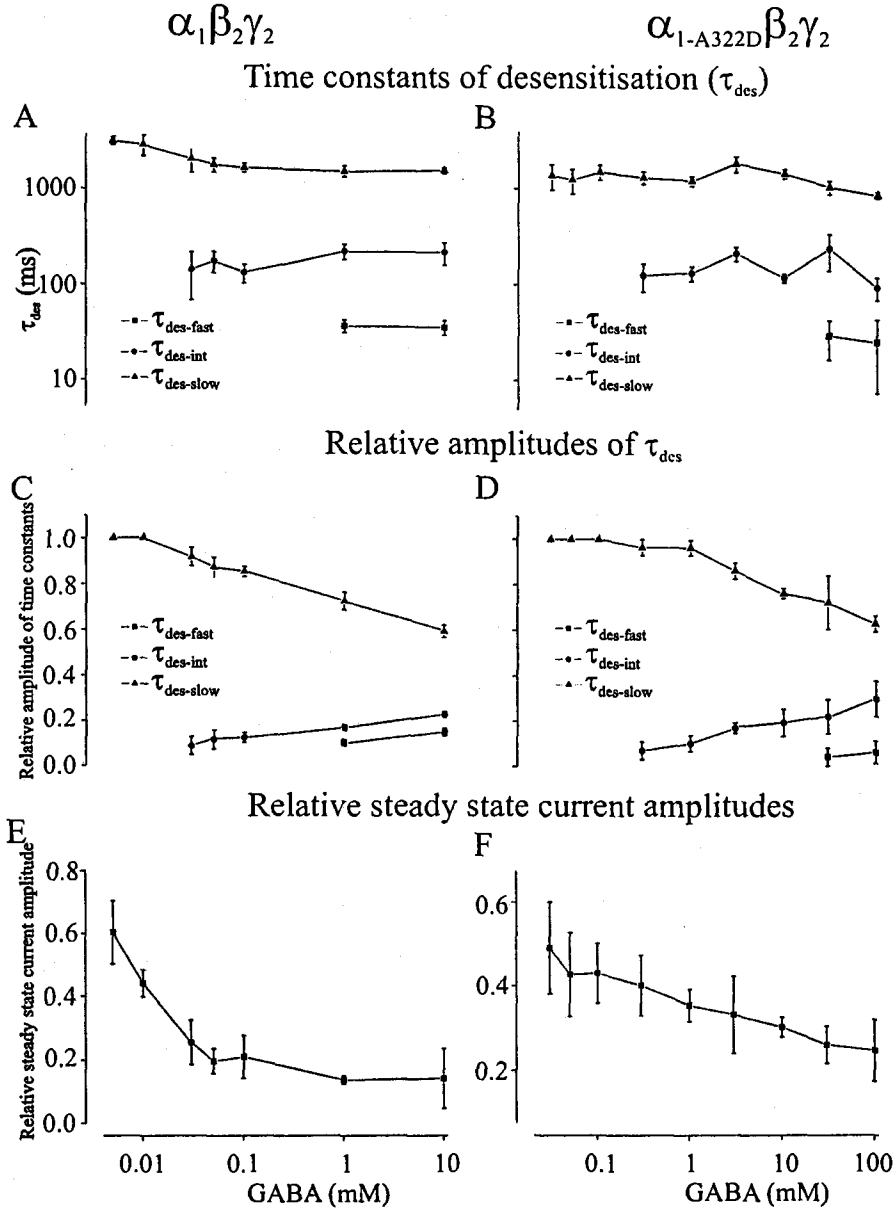


FIG. 4. Dose dependence of $GABA_A$ receptor desensitization. (A and B) Time constants of desensitization as a function of the GABA concentration. (C and D) Relative amplitudes of time constants of desensitization. (E and F) Relative steady-state current amplitudes. Note the different concentration range of GABA plotted for WT and A322D mutant receptor channels. There is an obvious shift of desensitization parameters for mutant receptors towards higher GABA concentrations paralleling that of peak current amplitudes and current rise times (Figs 1 and 2).

difference in the relative amplitude of the steady-state current relative to the peak current. When recordings with 30 mM GABA of the mutant were compared to those with 1 mM for the WT receptor, we did not observe significant differences in any of the parameters (Table 1). Thus, the main change of the A322D mutation on desensitization kinetics is a marked rightward shift of the concentration dependence without affecting desensitization kinetics *per se* (Figs 4A–D and 5A, Table 1).

Kinetics of deactivation

The current decay recorded from $GABA_A$ receptors after removal of the agonist is defined as deactivation. It reflects a complex process involving unbinding of the agonist, gating, and desensitization (Jones & Westbrook, 1995, 1996). As the time course of deactivation is most closely related to the shape of the IPSC (Jones & Westbrook, 1995), changed deactivation properties are probably more important for the pathophysiological role of the A322D mutation within the α_1-subunit than the altered desensitization parameters described above. The time course of deactivation was determined after 1-ms-lasting applications of saturating concentrations of GABA, i.e. 1 or 30 mM GABA for WT or A322D mutant channels, respectively. Raw current traces are presented in Fig. 5B. A second order exponential function was best fitted to the current decay yielding two time constants of deactivation, $\tau_{deact\text{-}fast}$ and $\tau_{deact\text{-}slow}$, as shown by statistical comparison of the standard deviations of the respective mono- and biexponential fits.

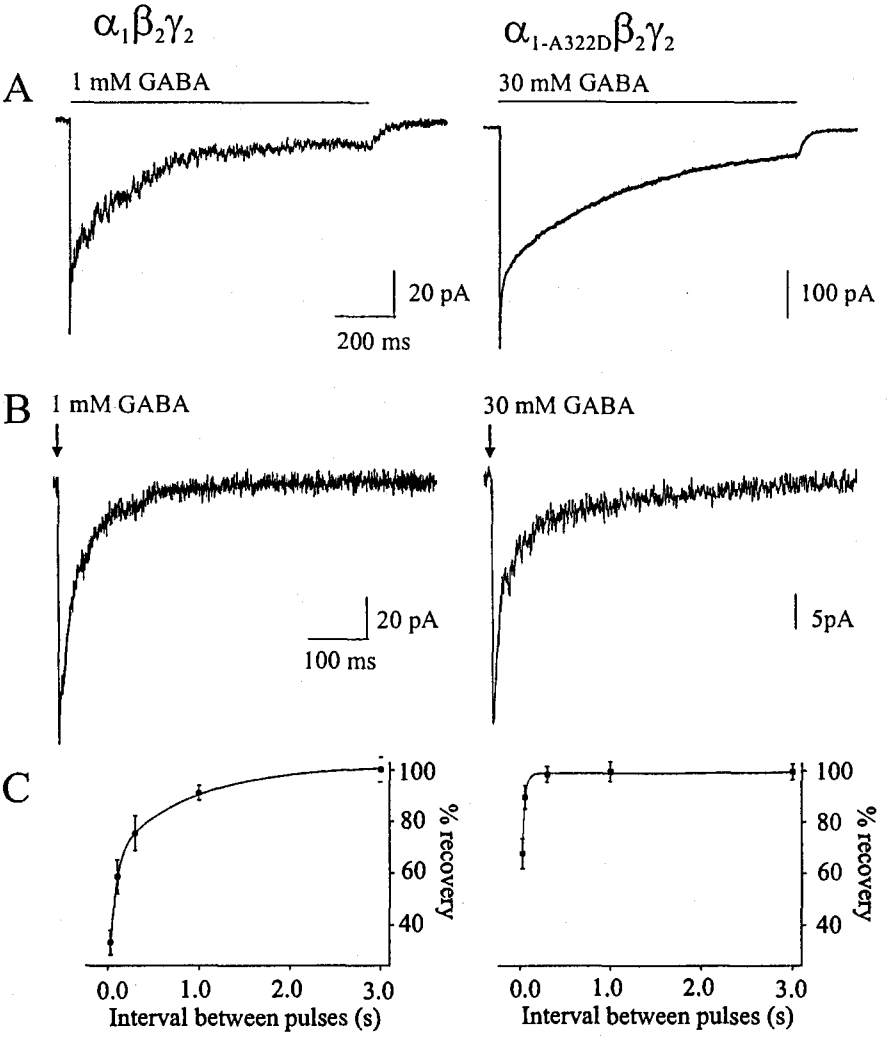


FIG. 5. Ultrafast agonist application experiments using outside-out patches. 1 mM GABA was used for WT, and 30 mM GABA for mutant receptors. The holding potential was −60 mV. (A) Time course of desensitization at saturating agonist concentrations. The current decay in the presence of the agonist is best described by the sum of three exponential functions. The desensitization parameters are similar at saturating GABA concentrations for WT (1 mM GABA) and A322D mutant receptors (30 mM GABA). The following time constants were obtained for the raw current traces shown. WT (left) $\tau_{des\text{-}fast}$ = 6.4 ms $\tau_{des\text{-}int}$ = 241 ms, $\tau_{des\text{-}slow}$ = 921 ms; A322D (right) $\tau_{des\text{-}fast}$ = 14.9 ms, $\tau_{des\text{-}int}$ = 147 ms, $\tau_{des\text{-}slow}$ = 844 ms. The average values obtained from all experiments are given in Table 1. (B) Time course of deactivation after short applications of a saturating agonist concentration (the time point of the 1-ms-lasting application is indicated by arrows). The fast and slow time constants describing the biexponential time course of deactivation were as follows: WT (left) $\tau_{deact\text{-}fast}$ = 21.5 ms, $\tau_{deact\text{-}slow}$ = 165 ms; A322D (right) $\tau_{deact\text{-}fast}$ = 7.3 ms, $\tau_{deact\text{-}slow}$ = 75 ms (compare to the average values in Table 1). For A322D mutant receptors, both components were approximately two to three times faster. Approximately two-thirds of the current decay was carried by the faster time constant in both receptor types. (C) Resensitization kinetics after 5-ms pulses of a saturating agonist concentration as studied by double pulse application with intervals of 30, 100, 300, 1000, and 3000 ms. A single exponential (τ_{res} = 45 ms) was fit to the average time course of relative resensitization recorded for mutant receptors (right), whereas a biexponential time course ($\tau_{res\text{-}fast}$ = 64 ms, $\tau_{res\text{-}slow}$ 544 ms) was found for the WT (left).

Their mean values as determined in the outside-out configuration are presented in Table 1. Deactivation was considerably accelerated for mutant compared to WT channels as both time constants were significantly shorter ($P = 0.010$ for $\tau_{deact\text{-}fast}$ and $P = 0.019$ for $\tau_{deact\text{-}slow}$). Compared to whole cell experiments with small cells lifted from the bottom (not shown), $\tau_{deact\text{-}fast}$ and $\tau_{deact\text{-}slow}$ were between four- and eight-times faster when determined from recordings of outside-out patches, and the relative amplitudes of the faster component were significantly larger (data not shown).

Kinetics of resensitization

Resensitization of $GABA_A$ receptors can be experimentally determined in paired pulse inhibition experiments. It gives a measure for the recovery of the channels from desensitized states after cessation of an agonist pulse. Resensitization was analysed in outside-out patches using 5-ms pulses of 1 mM GABA for WT and of 30 mM for mutant receptors and intervals between pulses of 30, 100, 300, 1000, and 3000 ms (Fig. 5C). Averaged data from 11 and 13 independent experiments for WT and A322D mutant channels, respectively, were analysed. For the WT, a second order exponential function was best fit to the averaged time course of relative resensitization, yielding $\tau_{res\text{-}fast} = 64$ ms and $\tau_{res\text{-}slow} = 544$ ms. The relative amplitude of $\tau_{res\text{-}fast}$ was 0.49. For A322D mutant channels, resensitization was much faster and followed a monoexponential time course with $\tau_{res\text{-}fast} = 45$ ms. Introduction of an additional slower exponential did not significantly reduce the standard deviation of the fit.

Activation by phenobarbital

From the experiments performed so far, it cannot be decided if the large rightward shift of the GABA dose–response curves results (i) from a reduced binding affinity of GABA to mutant channels or (ii) from gating changes after binding of GABA mediating the opening of the channel. Therefore, we conducted experiments with another agonist, the barbiturate phenobarbital that has a binding site distinct from that of GABA (Amin & Weiss, 1993). Upon application of phenobarbital to whole cells expressing $\alpha_1\beta_2\gamma_2$ human $GABA_A$ receptors current transients with kinetics different from that of GABA-activated currents were observed (Rho *et al.*, 1996; Akk & Steinbach, 2000; Krampfl *et al.*, 2002a). In the present study, application of 100 μM phenobarbital resulted in a small, nondesensitizing current for WT as well as for A322D mutant receptors (Fig. 6). As previously described, application of phenobarbital concentrations ≥300 μM resulted in a rebound current after removal of phenobarbital, while the maximum current amplitude in the presence of phenobarbital showed a bell-shaped dose–response curve with a decrease of current amplitudes at concentrations >1 mM (Wooltorton *et al.*, 1997; Krampfl *et al.*, 2002a). The maximum peak current amplitude of the rebound current was chosen to determine the dose–response relation for the activation of WT and mutant A322D $GABA_A$ receptors by phenobarbital (six independent experiments for each, WT and A322D mutant channels). We did not observe a significant difference between WT and mutant receptors, with an EC_{50} of 2.1 ± 0.3 mM and 2.5 ± 0.2 mM, respectively (Fig. 6).

Surface expression of EGFP-tagged constructs

To find out the reason for the reduced current density observed for mutant A322D compared to WT receptors, we constructed fusion proteins of both the mutant and WT α_1-subunit of the $GABA_A$ receptor with the enhanced green fluorescent protein (EGFP) (see Materials and methods). Transfection of the EGFP-tagged constructs together with β_2- and γ_2-subunits into HEK293 cells combined with confocal laser-scanning microscopy and overlay with a red-fluorescent membrane marker (FM4-64) revealed a pronounced staining of the surface membrane for the WT (EGFP-α_1) indicating that this channel is regularly integrated into the cell membrane (Fig. 7, upper row). GABA-induced currents could be regularly obtained from transfected cells, however, with smaller mean current amplitudes (304 ± 74 pA, $n = 10$) compared to the WT without EGFP

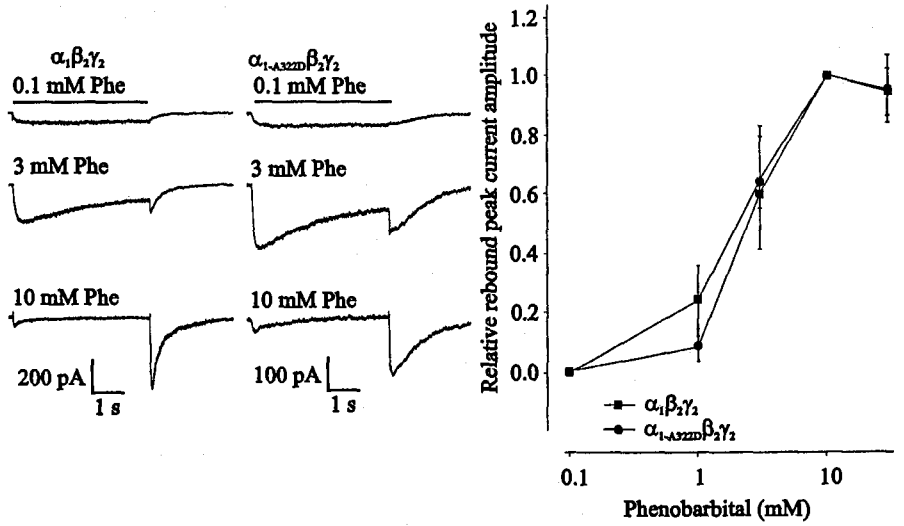


FIG. 6. Response to phenobarbital. Raw current traces recorded upon application of different concentrations of phenobarbital for WT and mutant receptors, respectively. Phenobarbital elicits a small nondesensitizing current at a concentration of 0.1 mM. At higher concentrations, phenobarbital not only activated but also blocked the receptor resulting in a rebound current upon removal of the agonist, which increased in amplitude with increasing phenobarbital concentrations. Dose dependence of rebound current amplitudes after application of phenobarbital for WT and mutant $GABA_A$ receptor channels. The EC_{50} determined for these rebound currents was not significantly different between mutant and WT $GABA_A$ receptor channels (the exact EC_{50} values are given in the text).

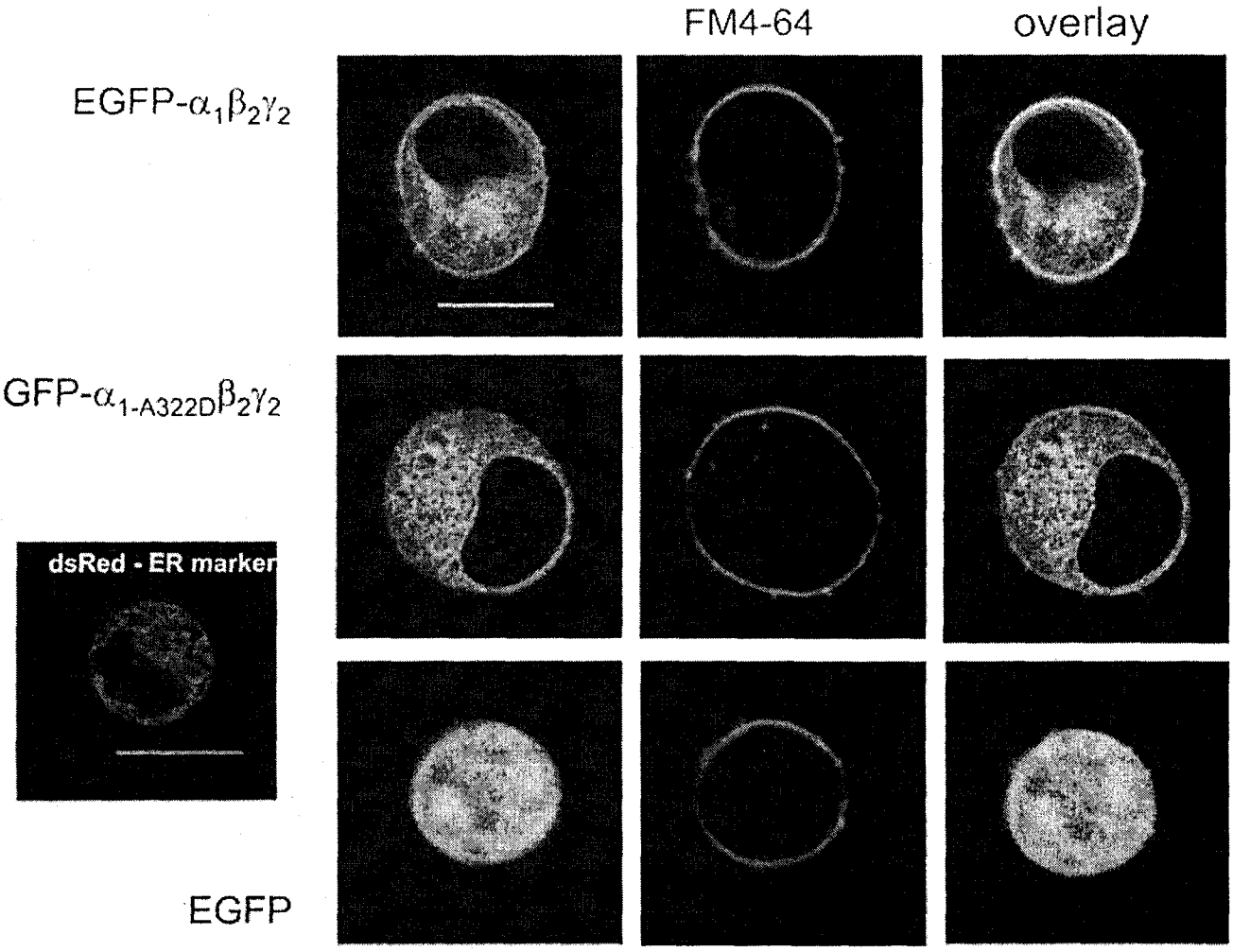


FIG. 7. Expression of EGFP-fusion proteins. HEK293 cells were cotransfected with either EGFP-$\alpha_1/\beta_2/\gamma_2$, EGFP-$\alpha_{1\text{-}A322D}/\beta_2/\gamma_2$, or EGFP alone and visualized using laser-scanning microscopy. EGFP was excited with the 488-nm line of the argon ion laser. For detection of fluorescence we used band-pass filter 515/30 nm. Membrane specific labelling was performed by adding FM4-64 to the medium used for live confocal imaging (HeNe laser, excitation 543 nm, emission >570 nm). The overlay shows a clear localization in the surface membrane for WT (upper row), but not for mutant $GABA_A$ receptors (middle row). Shown are representative images from minimum 100 cells observed for each transfection group in at least three different transfections. A control experiment with EGFP alone showed a homogeneous staining of the cytoplasm and the nucleus, but not the membrane (lower row). Cells transfected with dsRed2-ER (inset in the left), a marker for the endoplasmatic reticulum (ER), showed a similar structured fluorescence pattern to those transfected with mutant or WT EGFP-labelled receptors, but not to those transfected with EGFP alone. Scale bars, 10 μm.

(624 ± 180 pA, $n = 16$). In contrast, we could not detect any staining of the cell surface membrane for the mutant receptor (EGFP-$\alpha_{1\text{-}A322D}$). The green fluorescence seemed to be structured over the cytoplasm and the nucleus was spared (Fig. 7, middle row) – similar to that observed for dsRed2-ER, a marker for the endoplasmic reticulum (ER) (Fig. 7, inset on the left) – whereas for EGFP alone (Fig. 7, lower row) a quite homogeneous distribution all over the cell including the nucleus was found. In electrophysiological experiments, no currents could be recorded from those cells transfected with EGFP-$\alpha_{1\text{-}A322D}$, β_2 and γ_2 in a 1 : 1 : 2 ratio. In previous experiments, Western blots have shown that the mutant protein is regularly produced (Cossette *et al.*, 2002). This could be confirmed in our study for the fusion proteins EGFP-α_1 and EGFP-$\alpha_{1\text{-}A322D}$ using a commercially available polyclonal antibody against EGFP, as for both constructs, but not for nontransfected cells, we could detect a band of the expected molecular weight of approximately 78 kDa (results not shown).

These data indicate a reduced surface expression of the EGFP-tagged constructs *per se*, which showed reduced current amplitudes compared to the native channels without EGFP. Nevertheless, there was a fundamental difference between WT and mutant receptors in surface expression, which can explain the reduced amplitudes recorded for mutant receptors in patch clamp experiments. The structured staining of EGFP-$\alpha_{1\text{-}A322D}$ constructs resembling the fluorescence in cells transfected with dsRed2-ER marker, compared to the homogeneous staining for EGFP alone, might indicate that mutant receptors are retained in the ER and are not transported to the surface membrane.

Discussion

Pathophysiology of idiopathic generalized epilepsies

In the presented study, we compared the gating parameters and the expression of WT and mutant A322D $GABA_A$ receptor α_1-subunits

when coexpressed with β_2- and γ_2-subunits in a heterologous expression system, HEK293 cells, using a combination of electrophysiological and morphological techniques. Our results clearly reveal a loss-of-function of the mutant receptor as the disease-causing mechanism for juvenile myoclonic epilepsy. In particular, we have shown that the severe loss-of-function is produced by a combination of different molecular pathomechanisms, including reduced surface expression, reduced GABA-sensitivity, and accelerated deactivation. As in previous studies using recombinant receptors bearing mutations in either *GABRA1* (Cossette *et al.*, 2002; Fisher, 2004), or *GABRG2* (Baulac *et al.*, 2001; Wallace *et al.*, 2001; Harkin *et al.*, 2002; Kananura *et al.*, 2002), the occurrence of epileptic seizures can be explained well by an impairment of GABA-ergic transmission. Interestingly, various mutations in the chloride channel ClC-2 are also associated with idiopathic generalized epilepsies (Haug *et al.*, 2003). This voltage-gated chloride channel plays a role in establishing and maintaining the high transmembrane chloride gradient, which is essential for an inhibitory GABA response. Therefore, there is increasing evidence supporting the view that a disturbed GABAergic inhibition is as a central pathophysiological mechanism for these common forms of epilepsy (Mulley *et al.*, 2003; Noebels, 2003; Lerche *et al.*, 2004).

Molecular pathophysiology of the A322D mutation

The main molecular mechanisms of the A322D mutation leading to a loss-of-function of the pentameric receptor were a markedly reduced macroscopic current amplitude at saturating GABA concentrations and a 30- to 40-fold reduction in GABA sensitivity. Biochemical and morphological experiments showed that the mutant protein was produced regularly and probably located in the ER, but less inserted into the surface membrane compared to the WT receptor. As the single channel conductance and current-voltage relation were also unchanged in the mutant receptor, our data indicate that a reduced surface expression accounts for the observed reduction in current amplitude. Beside the reduction in surface expression, the biophysical defects cause a severe loss-of-function of the mutant receptor by a reduced GABA-dependent current amplitude, a slower rising phase, and an accelerated deactivation of GABA-ergic currents, which most likely will markedly reduce and shorten the resulting IPSCs. $GABA_A$ receptor subunits are highly conserved. The A322D mutation of the α_1-subunit, which leads to a special form of JME, is a dominant allele. As shown in Fig. 1C, when mutant and native α_1-subunits were cotransfected with β_2- and γ_2-subunits, the dose–response curve gave an intermediate EC_{50}. From these data it might be assumed that there is no interaction (like, e.g. a dominant-negative effect) between mutant and native α_1-subunits.

Potential biophysical mechanism of impaired channel gating by the A322D mutation

The question remains, as to how the A322D mutation alters channel gating on a molecular level. As the mutation is located deeply within in third transmembrane domain (M3), far away from the extracellularly located putative binding site for GABA (Mehta & Ticku, 1999; Sieghart & Sperk, 2002; Kash *et al.*, 2004), it is likely that the reduced GABA sensitivity of the mutant receptor occurs as a result of partial uncoupling of GABA binding to the opening of the gate. This coupling mechanism is not well understood up to now, but the M2–M3 extracellular linker has been suggested to contribute to transmit ligand-binding into an opening of the gate, which could be formed by the M2 segment (Kash *et al.*, 2004). Thus, the M3 segment may play a role in coupling as well, which could be influenced by the A322D mutation via inducing a conformational change of M3 and disturbing a M3–M2 interaction, thereby uncoupling agonist binding from channel gating. As such a putative role for the M3 segment has not been suggested up to now, this is another example of how disease-causing mutations can point to protein regions important for channel gating, helping to understand the molecular determinants of ion channel function (Lehmann-Horn & Jurkat-Rott, 1999).

Our experiments using a barbiturate as another $GABA_A$ receptor agonist, which has a different binding site than GABA (Amin & Weiss, 1993), did not show a significant difference between WT and mutant receptor activation (Fig. 6). Thus, the coupling mechanism of barbiturate binding to channel opening is obviously different from the one for GABA. As channel gating functioned in a normal way under these conditions, these data indicate that the gate itself is not affected by the A322D mutation. The normal single channel conductance and current–voltage relationship confirm that pore properties are not altered, as was also found for the corresponding mutation in the rat isoform of this receptor (Fisher, 2004). Our data thus strongly suggest, that the A322D mutation changes the coupling between GABA binding and opening of the receptors gate quite specifically, while there seems to be no significant change in channel gating itself and activation by barbiturates.

Acknowledgements

This article is dedicated to the memory of Leonid Komissarow. We thank Drs W. Melzer, D. Ursu, and O. Wittekind for introducing us in the confocal imaging technique and for their advice, U. Jensen for help with transfection and maintaining the cell culture, A. Niesl, J. Kilian, and A. Riecker for further technical support. This work was supported by grants of the Deutsche Forschungsgemeinschaft (to JB and KK, DFG Bu938/8–1; to HL, DFG Le1030/9–1), the Volkswagenstiftung (to HL), and the Bundesministerium für Bildung und Forschung (to HL, NGFN2/TP854). HL is a Heisenberg fellow of the DFG.

Abbreviations

EGFP, enhanced green fluorescent protein; HEK, human embryonic kidney; IGE, idiopathic generalized epilepsies; IPSCs, inhibitory postsynaptic currents; JME, juvenile myoclonic epilepsy; WT, wild-type.

References

Akk, G. & Steinbach, J.H. (2000) Activation and block of recombinant GABA(A) receptors by pentobarbitone: a single-channel study. *Br. J. Pharmacol.*, **130**, 249–258.

Amin, J. & Weiss, D.S. (1993) $GABA_A$ receptor needs two homologous domains of the beta-subunit for activation by GABA but not by pentobarbital. *Nature*, **366**, 565–569.

Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'Homme, J.F., Baulac, M., Brice, A., Bruzzone, R. & LeGuern, E. (2001) First genetic evidence of $GABA_A$ receptor dysfunction in epilepsy: a mutation in γ_2-subunit gene. *Nature Genet.*, **28**, 46–48.

Bianchi, M.T., Song, L., Zhang, H. & Macdonald, R.L. (2002) Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. *J. Neurosci.*, **22**, 5321–5327.

Clements, J.D., Feltz, A., Sahara, Y. & Westbrook, G.L. (1998) Activation kinetics of AMPA receptor channels reveal the number of functional agonist binding sites. *J. Neurosci.*, **18**, 119–127.

Cossette, P., Liu, L., Brisebois, K., Dong, H., Lortie, A., Vanasse, M., Saint-Hilaire, J.M., Verner, A., Lu, W.Y., Wang, Y.T. & Rouleau, G.A. (2002)

Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nature Genet.*, **31**, 184–189.

Fisher, J.L. (2004) A mutation in the $GABA_A$ receptor α1 subunit linked to human epilepsy affects channel gating properties. *Neuropharmacology*, **46**, 629–637.

Franke, C., Hatt, H. & Dudel, J. (1987) Liquid filament switch for ultra-fast exchanges of solutions at excised patches of synaptic membrane of crayfish muscle. *Neurosci. Lett.*, **77**, 199–204.

Franke, C., Parnas, H., Hovav, G. & Dudel, J. (1993) A molecular scheme for the reaction between acetylcholine and nicotinic channels. *Biophys. J.*, **64**, 339–356.

Haas, K.F. & Macdonald, R.L. (1999) $GABA_A$ receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant $GABA_A$ receptor currents in mouse fibroblasts. *J. Physiol. (Lond.)*, **514**, 27–45.

Harkin, L.A., Bowser, D.N., Dibbens, L.M., Singh, R., Phillips, F., Wallace, R.H., Richards, M.C., Williams, D.A., Mulley, J.C., Berkovic, S.F., Scheffer, I.E. & Petrou, S. (2002) Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am. J. Hum. Genet.*, **70**, 530–536.

Haug, K., Warnstedt, M., Alekov, A.K., Sander, T., Ramirez, A., Poser, B., Maljevic, S., Hebeisen, S., Kubisch, C., Rebstock, J., Horvath, S., Hallmann, K., Dullinger, J.S., Rau, B., Haverkamp, F., Beyenburg, S., Schulz, H., Janz, D., Giese, B., Muller-Newen, G., Propping, P., Elger, C.E., Fahlke, C., Lerche, H. & Heils, A. (2003) Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nature Genet.*, **33**, 527–532.

Jones, M.W. & Westbrook, G.L. (1995) Desensitized states prolong $GABA_A$ channel responses to brief agonist pulses. *Neuron*, **15**, 181–191.

Jones, M.W. & Westbrook, G.L. (1996) The impact of receptor desensitisation on fast synaptic transmission. *TINS*, **19**, 96–101.

Kananura, C., Haug, K., Sander, T., Runge, U., Gu, W., Hallmann, K., Rebstock, J., Heils, A. & Steinlein, O.K. (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch. Neurol.*, **59**, 1137–1141.

Kash, T.L., Trudell, J.R. & Harrison, N.L. (2004) Structural elements involved in activation of the γ-aminobutyric acid type A ($GABA_A$) receptor. *Biochem. Soc. Transact.*, **32**, 540–546.

Kittler, J.T., Wang, J., Connolly, C.N., Vicini, S., Smart, T.G. & Moss, S.J. (2000) Analysis of $GABA_A$ receptor assembly in mammalian cell lines and hippocampal neurons using γ_2-subunit green fluorescent protein chimeras. *Mol. Cell. Neurosci.*, **16**, 440–452.

Krampfl, K., Bufler, J., Lepier, A., Dudel, J. & Adelsberger, H. (2000) Desensitisation characteristics of rat recombinant $GABA_A$ receptors consisting of $\alpha_1\beta_2\gamma_{2S}$ and $\alpha_1\beta_2$ subunits expressed in HEK293 cells. *Neurosci. Lett.*, **278**, 21–24.

Krampfl, K., Schlesinger, F., Zörner, A., Kappler, M., Dengler, R. & Bufler, J. (2002b) Control of kinetic properties of GluR2 flop AMPA-type channels: impact of R/G nuclear editing. *Eur. J. Neurosci.*, **15**, 51–62.

Krampfl, K., Wolfes, H., Dengler, R. & Bufler, J. (2002a) Kinetic analysis of the agonistic and blocking properties of pentobarbital on recombinant rat alpha(1) beta(2) gamma(2S) GABA(A) receptor channels. *Eur. J. Pharmacol.*, **435**, 1–8.

Lehmann-Horn, F. & Jurkat-Rott, K. (1999) Voltage-gated ion channels and hereditary disease. *Physiol. Rev.*, **79**, 1317–1372.

Lerche, H., Weber, Y.G., Jurkat-Rott, K. & Lehmann-Horn, F. (2004) Ion channel defects in idiopathic epilepsies. *Curr. Pharmaceut. Design.*, in press.

Mehta, M.K. & Ticku, M.K. (1999) An update on $GABA_A$ receptors. *Brain Res. Brain Res. Rev.*, **29**, 196–217.

Mohammadi, B., Krampfl, K., Cetinkaya, C., Moschref, H., Grosskreutz, J., Dengler, R. & Bufler, J. (2003) Kinetic analysis of recombinant mammalian alpha(1) and alpha(1) beta glycine receptor channels. *Eur. Biophys. J.*, **32**, 529–536.

Mohler, H., Fritschy, J.M., Lüscher, B., Rudolph, U., Benson, J. & Benke, D. (1996) The $GABA_A$ receptors: from subunits to diverse functions. *Ion Channels*, **4**, 89–113.

Mulley, J.C., Scheffer, I.E., Petrou, S. & Berkovic, S.F. (2003) Channelopathies as a genetic cause of epilepsy. *Curr. Opin. Neurol.*, **16**, 171–176.

Noebels, J.F. (2003) The biology of epilepsy genes. *Annu. Rev. Neurosci.*, **26**, 599–625.

Partin, K.M., Fleck, M.W. & Mayer, M.L. (1996) AMPA receptor flip/flop mutants affecting deactivation, desensitization, and modulation by cyclothiazide, aniracetam, and thiocyanate. *J. Neurosci.*, **16**, 6634–6647.

Ramakrishnan, L. & Hess, G.P. (2004) On the mechanism of a mutated and abnormally functioning gamma-aminobutyric acid (A) receptor linked to epilepsy. *Biochemistry*, **43**, 7534–7540.

Ratner, E., Tour, O. & Parnas, H. (2000) Evaluation of the number of agonist molecules needed to activate a ligand-gated channel from the current rising phase. *Biophys. J.*, **78**, 731–745.

Rho, J.M., Donevan, S.D. & Rogawski, M.A. (1996) Direct activation of $GABA_A$ receptors by barbiturates in cultured rat hippocampal neurons. *J. Physiol. (Lond.)*, **497**, 509–522.

Schlesinger, F., Meywirth, J., Krampfl, K., Grosskreutz, J., Petri, S., Mauth, C., Just, L., Bader, A. & Bufler, J. (2004) Ligand-gated channels in early mesencephalic neuronal precursors: immunocytochemical and electrophysiological analysis. *Eur. J. Neurosci.*, **19**, 2371–2376.

Sieghart, W. & Sperk, G. (2002) Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr. Top. Med. Chem.*, **2**, 795–816.

Wallace, R.H., Marini, C., Petrou, S., Harkin, L.A., Bowser, D.N., Panchal, R.G., Williams, D.A., Sutherland, G.R., Mulley, J.C., Scheffer, I.E. & Berkovic, S.F. (2001) Mutant $GABA_A$ receptor γ_2-subunit in childhood absence epilepsy and febrile seizures. *Nature Genet.*, **28**, 49–52.

Wooltorton, J.R., Moss, S.J. & Smart, T.G. (1997) Pharmacological and physiological characterization of murine homomeric beta3 GABA(A) receptors. *Eur. J. Neurosci.*, **9**, 2225–2235.

APPENDIX 2: A MUTATION IN THE $GABA_A$ RECEPTOR α1-SUBUNIT IS ASSOCIATED WITH ABSENCE EPILEPSY

Reference :

Maljevic S, Krampfl K, Cobilanschi J, Tilgen N, Beyer S, Weber YG, Schlesinger F, Ursu D, Melzer W, Cossette P, Bufler J, Lerche H, and Heils A. A mutation in the $GABA_A$ receptor α1-subunit is associated with absence epilepsy. *Annals of Neurology* 2006; 59: 983-987.

References

1. Rowland LP. Hereditary and acquired motor neuron diseases. In: Rowland LP, ed. Merritt's textbook of neurology. 9 ed. Philadelphia: Williams & Wilkins, 1995:742–749.
2. Julien JP. Amyotrophic lateral sclerosis: unfolding the toxicity of the misfolded. Cell 2001;104:581–591.
3. Almer G, Guégan C, Teismann P, et al. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. Ann Neurol 2001;49:176–185.
4. Maihofner C, Probst-Cousin S, Bergmann M, et al. Expression and localization of cyclooxygenase-1 and -2 in human sporadic amyotrophic lateral sclerosis. Eur J Neurosci 2003;18: 1527–1534.
5. Yasojima K, Tourtellotte WW, McGeer EG, et al. Marked increase in cyclooxygenase-2 in ALS spinal cord: implications for therapy. Neurology 2001;57:952–956.
6. Drachman DB, Rothstein JD. Inhibition of cyclooxygenase-2 protects motor neurons in an organotypic model of amyotrophic lateral sclerosis. Ann Neurol 2000;48:792–795.
7. Drachman DB, Frank K, Dykes-Hoberg M, et al. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. Ann Neurol 2002; 52:771–778.
8. Kiaei M, Kipiani K, Petri S, et al. Integrative role of cPLA with COX-2 and the effect of non-steriodal anti-inflammatory drugs in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurochem 2005;93:403–411.
9. Almer G, Teismann P, Stevic Z, et al. Increased levels of the pro-inflammatory prostaglandin PGE2 in CSF from ALS patients. Neurology 2002;58:1277–1279.
10. Kostic V, Jackson-Lewis V, De Bilbao F, et al. Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. Science 1997;277:559–562.
11. Azari MF, Profyris C, Le Grande MR, et al. Effects of intraperitoneal injection of Rofecoxib in a mouse model of ALS. Eur J Neurol 2005;12:357–364.
12. Klivenyi P, Kiaei M, Gardian G, et al. Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurochem 2004;88:576–582.
13. Bilak M, Wu L, Wang Q, et al. PGE2 receptors rescue motor neurons in a model of amyotrophic lateral sclerosis. Ann Neurol 2004;56:240–248.
14. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–182.

A Mutation in the $GABA_A$ Receptor α_1-Subunit Is Associated with Absence Epilepsy

Snezana Maljevic, PhD,[1,2] Klaus Krampfl, MD,[3] Joana Cobilanschi, PhD,[4] Nikola Tilgen, MD,[4] Susanne Beyer, BS,[4] Yvonne G. Weber, MD,[1] Friedrich Schlesinger, MD,[3] Daniel Ursu, PhD,[2] Werner Melzer, PhD,[2] Patrick Cossette, MD, PhD,[5] Johannes Bufler, MD,[3] Holger Lerche, MD,[1,2]* and Armin Heils, MD[4,6]*

Objective: To detect mutations in *GABRA1* in idiopathic generalized epilepsy.

Methods: GABRA1 was sequenced in 98 unrelated idiopathic generalized epilepsy patients. Patch clamping and confocal imaging was performed in transfected mammalian cells.

Results: We identified the first *GABRA1* mutation in a patient with childhood absence epilepsy. Functional studies showed no detectable GABA-evoked currents for the mutant, truncated receptor, which was not integrated into the surface membrane.

Interpretation: We conclude that this de novo mutation can contribute to the cause of "sporadic" childhood absence epilepsy by a loss of function and haploinsufficiency of the $GABA_A$ receptor α_1-subunit, and that *GABRA1* mutations rarely are associated with idiopathic generalized epilepsy.

Ann Neurol 2006;59:983–987

Recent studies have shown that genetically driven ion channel dysfunction plays a major role in the pathophysiology of rare inherited idiopathic epilepsies. In particular, inhibitory GABAergic neurotransmission appears to be affected in these diseases.[1,2] Recently, two of the main $GABA_A$ receptor subunit genes have been identified to be associated with different inherited

From the [1]Neurologische Klinik; [2]Abteilung für Angewandte Physiologie, Universität Ulm, Ulm; [3]Neurologische Klinik, Medizinische Hochschule Hannover, Hannover; [4]Klinik für Epileptologie, Universität Bonn, Bonn, Germany; [5]Département de Médecine, Université de Montréal, CHUM-Hôpital Notre-Dame Montréal, Montréal, Québec, Canada; and [6]Institut für Humangenetik, Universität Bonn, Bonn, Germany.

H.L. and A.H. are both corresponding authors.

Received Oct 6, 2005, and in revised form Mar 21, 2006. Accepted for publication Mar 24, 2006.

Published online May 22, 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20874

Address correspondence to Dr Heils, Klinik für Epileptologie und, Institut für Humangenetik, Universitätsklinikum Bonn,Sigmund-Freud-Strasse 25, 53105 Bonn, Germany.
E-mail: armin.heils@ukb.uni-bonn.de or holger.lerche@uni-ulm.de

epileptic disorders. Mutations in *GABRG2*, encoding the γ_2-subunit, were found in families with generalized epilepsy with febrile seizures plus ($GEFS^+$) and childhood absence epilepsy (CAE) with febrile seizures,[3–6] whereas a mutation in *GABRA1*, encoding the α_1-subunit, has been detected in a family with juvenile myoclonic epilepsy.[7] To investigate whether *GABRA1* mutations contribute to the cause of other common forms of IGE, we sequenced this gene in 98 unrelated IGE patients.

Subjects and Methods

Study Sample

Ninety-eight patients (58 male and 40 female patients) of German origin diagnosed[8] with 1 of the 4 main IGE syndromes, including juvenile myoclonic epilepsy (n = 30), CAE (n = 38), juvenile absence epilepsy (n = 19), and epilepsy with grand-mal seizures on awakening (n = 11), were included in our study, which was approved by the Ethics Committee of the University of Bonn. Sixty patients had a family history with at least 1 first-degree relative affected with IGE, and 38 were sporadic cases. Written informed consent was obtained from all participants. Two hundred ninety-two healthy individuals of German descent served as control subjects.

The diagnosis of CAE in the mutation carrier was based on clinical interview and available medical records. His parents reported short episodes with a loss of consciousness occurring in typical pyknoleptic daily clusters between 3 and 5 years of age. There was no history of febrile seizures. Electroencephalogram recordings at 4 years of age showed 3/second spike-wave discharges provoked by hyperventilation associated with a short loss of consciousness. The patient was treated with valproate from the age of 5 years and remained seizure-free until now (current age, 18 years). There was no history of epilepsy or febrile seizures in the unaffected brother and both parents, according to interviews of the parents and both grandmothers of the index patient. Electroencephalogram recordings of the unaffected brother at 16 years of age and both parents were normal.

Genetic Studies and Mutagenesis

We amplified and sequenced all *GABRA1* coding exons and adjacent splice sites (primers are available on request). Maternity and paternity were ascertained by genotyping the following highly polymorphic STR markers: *D2S1266, D4S2293, D5S580, D6S395, D7S622, D8S396, D9S234, D10S518, D13S241, D14S549, D15S1356, D16S488, D17S1306, D18S1002, D22S526.*

The QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used to introduce the single base pair deletion (975delC) in the human complementary DNA of *GABRA1* in pcDNA3.1(−),[7] which was verified by sequencing the whole coding region. The enhanced green fluorescent protein (EGFP) was inserted between amino acids four and five of the mature human α_1-subunit.[9]

Cell Culture, Electrophysiology, Confocal Imaging, and Immunoblots

All procedures were performed as described previously.[9] Human embryonic kidney (HEK293) cells were transfected with the human wild-type (WT) or mutant α_1-subunit (denoted as α_1 or $\alpha_{1\text{-}S326fs328X}$, respectively), together with human β_2- and γ_2-subunits in a 1:1:2 ratio, if not indicated otherwise, using calcium-phosphate or Fugene 6 Transfection Reagent (Roche Applied Science, Mannheim, Germany) and analyzed after 24 to 48 hours. The GFP-labeled murine γ_2-subunit used for one subset of experiments was kindly provided by Dr P. Wulff.[10]

Standard whole-cell patch clamping was performed using an Axopatch 200B amplifier and *Axon* digital data acquisition (Axon Instruments, Union City, CA) combined with ultrafast application of GABA (0.1μM to 10mM). Patch pipettes were filled with "intracellular" solution (in mM): 140 KCl, 11 EGTA, 10 Hepes, 10 glucose, 2 $MgCl_2$. The bath solution contained (in mM): 162 NaCl, 5.3 KCl, 2 $CaCl_2$, 0.67 NaH_2PO_4, 0.22 KH_2PO_4, 15 Hepes, 5.6 glucose. The holding potential was kept between −40 and −80mV. All data are given as means ± standard error of the mean.

Images were obtained using a confocal laser-scanning microscope (Radiance 2000; Zeiss, Oberkochen, Germany). The membrane-specific dye, FM4-64 (Molecular Probes, Eugene, OR), was applied in a final concentration of 5μM. A plasmid for expression of the dsRed2-ER marker (Clontech, Palo Alto, CA) was (co)transfected. Immunocytochemistry on nonpermeabilized cells and immunoblot analysis were performed with polyclonal antibodies directed against EGFP (Chemicon, Temecula, CA) using cells transfected with WT or mutant EGFP-$\alpha_1\beta_2\gamma_2$ or their lysates (protocols are available on request).

Results

Mutation Screening

A single base pair deletion (975delC) predicting a frameshift and a premature stop codon (S326fs328X) was identified in *GABRA1* in one affected individual with CAE (Fig 1). Both parents and the unaffected brother carried two WT alleles (see Fig 1A). Paternity and maternity were confirmed (see Subject and Methods). The mutation thus arose de novo in this individual with "sporadic" CAE and was absent in 292 ethnically matched healthy control subjects. No other sequence variations were detected in *GABRA1* in any of the 98 affected patients investigated.

Functional Studies

Fast-activating and slowly desensitizing GABA-evoked currents were recorded for the WT receptor ($\alpha_1\beta_2\gamma_2$ in a 1:1:2 ratio),[9] but no currents were detected in cells transfected with $\alpha_{1\text{-}S326fs328X}\beta_2\gamma_2$ subunits (Fig 2). When mutant and WT α_1-subunits were cotransfected ($\alpha_1/\alpha_{1\text{-}S326fs328X}/\beta_2/\gamma_2$ in a 1:1:1:2 ratio), no significant dominant negative effect was observed, because current amplitudes were 681 ± 285 (n = 10) compared with 624 ± 180pA (n = 16) for the WT alone.

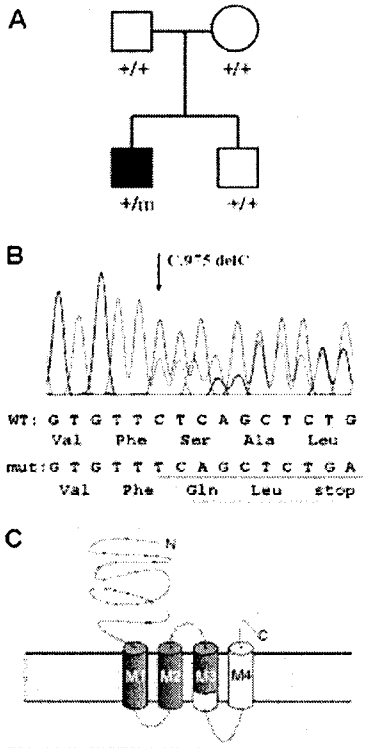


Fig 1. Pedigree and sequencing results illustrating a de novo mutation in GABRA1 *in an individual with sporadic childhood absence epilepsy (CAE). A single base pair deletion (975delC) was detected predicting a frameshift mutation and a premature stop codon (S326fs328X). (A) The pedigree indicates that the unaffected parents and one unaffected brother carried two wild-type (WT) alleles (+/+), whereas the index patient carried the mutation and one WT allele (+/m). (B) Sequencing results of a portion of* GABRA1, *showing one wild-type allele and the 975delC mutation in the affected individual. (C) Schematic presentation of the $GABA_A$ receptor α_1-subunit. The premature stop codon results in a truncation starting within the third transmembrane region (M3; white).*

Different steps of protein formation and targeting could be affected leading to the nonfunctional $GABA_A$ receptor. To assess the cellular fate of WT compared with mutant channels, we constructed fusion proteins of the α_1-subunit with EGFP, which yielded functional receptors without any gating abnormalities.[9,10] The recombinant receptors were coexpressed with fluorescent markers for either surface membrane or endoplasmic reticulum (ER), and their localization determined using laser-scanning confocal microscopy. Whereas a clear membrane staining could be observed for WT receptors, fluorescence for $\alpha_{1\text{-}S326fs328X}\beta_2\gamma_2$ was found only in the cytoplasm, indicating that the mutant channels were not integrated in the plasma membrane (Fig 3A). The same patterns occurred when the GFP-tagged murine γ_2-subunit[10] was cotransfected with human β_2-subunit and WT or mutant α_1-subunits (see Fig 3B), confirming that the mutant $\alpha_{1\text{-}S326fs328X}$ was responsible for impeding surface expression. We also used immunocytochemistry with transfected, nonpermeabilized cells and an antibody directed against the extracellularly inserted EGFP to verify the lack of surface expression for the mutant compared with the WT protein (see Fig 3C). Using immunoblot analysis, we detected the expected band for EGFP-α_1 (78kDa). In contrast, for EGFP-$\alpha_{1\text{-}S326fs328X}$, a weaker band of the expected size (65kDa) followed by a broad smear of smaller proteins was observed (see Fig 3D). Whereas the structured intracellular fluorescence pattern for WT receptors corresponded largely to the one obtained with dsRed2-ER, the more homogenous cytoplasmic distribution for mutant receptors resembled the one for EGFP alone, but without a nuclear fluorescence (see Fig 3E, compare also Fig 3A). In contrast, for mutation A322D, which yields functional channels with a 5-fold reduced current density and a 30- to 40-fold reduction in GABA sensitivity, we previously obtained clear bands in Western blots and a structured distribution of fluorescent fusion proteins within the cytoplasm, similar to the WT, but also no clear membrane staining.[7,9] These results suggest that the mutant A322D is not degraded but largely remains in the ER with a reduced transport to the surface membrane, whereas S326fs328X studied here does not reach the plasma membrane at all and is degraded.

Discussion

In this study, we report the identification and functional characterization of the first mutation in

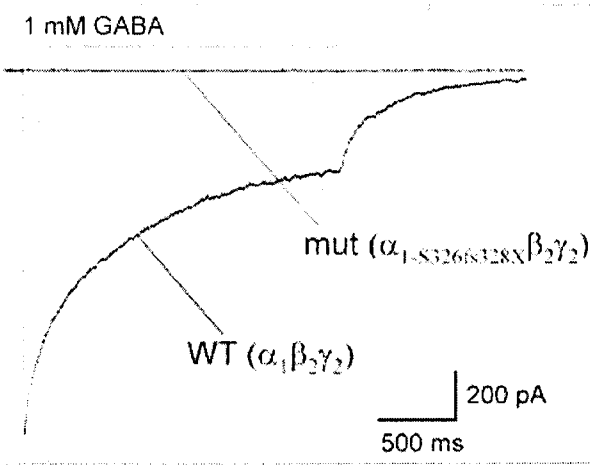


Fig 2. GABA-evoked currents of wild-type (WT) and mutated $GABA_A$ receptors. Complementary DNA encoding WT or mutant α_1-subunits were cotransfected into human embryonic kidney (HEK293) cells together with β_2- and γ_2-subunits. Whole-cell currents of $\alpha_1\beta_2\gamma_2$ and $\alpha_{1\text{-}S326fs328X}\beta_2\gamma_2$ were elicited by applying 1mM GABA to cells kept at a holding potential of −40mV. For the mutant construct, GABA-evoked currents could not be detected.

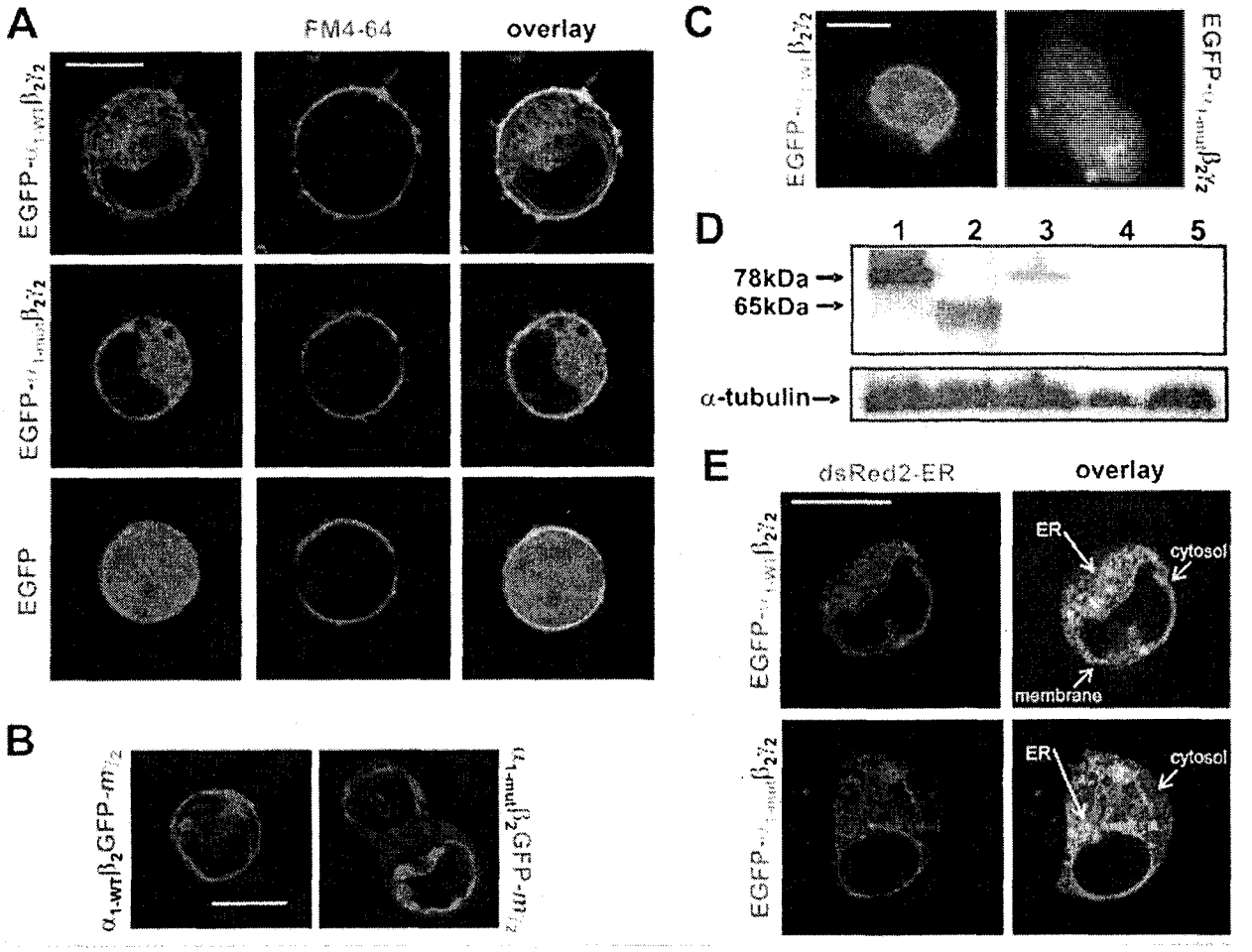


Fig 3. Expression of enhanced green fluorescent protein (EGFP) fusion proteins. Human embryonic kidney (HEK293) cells were cotransfected with either wild-type (WT) (EGFP-$\alpha_{1\text{-}WT}\beta_2\gamma_2$) or mutant constructs (EGFP-$\alpha_{1\text{-}mut}\beta_2\gamma_2$) or EGFP alone and visualized using laser-scanning microscopy. EGFP was excited with the 488nm line of the argon ion laser and detected using band-pass filter 515/30nm. (A) Membrane-specific labeling was performed by adding FM4-64 (5μM) to the medium used for live confocal imaging (HeNe laser; excitation, 543nm; emission, >570nm). The overlay showed clear membrane staining for the WT, but no surface expression for the mutant receptors. $n > 100$ for each construct. (B) Coexpression using the murine GFP-γ_2 with human α_1- and β_2-subunits shows membrane staining only for $\alpha_{1\text{-}WT}\beta_2$GFP-m$\gamma_2$, but not for $\alpha_{1\text{-}mut}\beta_2$GFP-m$\gamma_2$ cotransfections in HEK293 cells. $n > 30$. (C) Conventional fluorescent microscopic images of HEK293 cells expressing EGFP-$\alpha_{1\text{-}WT}\beta_2\gamma_2$ or EGFP-$\alpha_{1\text{-}mut}\beta_2\gamma_2$, protein complexes, incubated with a polyclonal antibody against EGFP before they were fixed, permeabilized, and their nuclei stained with 46'-diamidino-2-phenylindole-2 hydrochloride (DAPI) (blue). There was a clear staining of the surface membrane of cells transfected with WT receptors (left), which was never observed in those transfected with the mutant (right). $n > 50$. (D) Western blot analysis of the whole-cell lysates prepared from HEK293 cells transfected with the same total amount of the following complementary DNA: lane 1 = EGFP-$\alpha_{1\text{-}WT}$; lane 2 = EGFP-$\alpha_{1\text{-}mut}$; lane 3 = EGFP-$\alpha_{1\text{-}WT}\beta_2\gamma_2$; lane 4 = EGFP-$\alpha_{1\text{-}mut}\beta_2\gamma_2$. Lane 5 = nontransfected cells. Fusion proteins were detected using an anti-EGFP antibody. The bottom blot shows immunoreactivity to the intracellular protein α-tubulin, used as an internal loading control. The smear below the expected size of 65kDa for the mutant protein in lanes 2 and 4 could indicate degradation. (E) Representative cells coexpressing dsRed2-ER with EGFP-$\alpha_{1\text{-}WT}\beta_2\gamma_2$ or EGFP-$\alpha_{1\text{-}mut}\beta_2\gamma_2$. dsRed2 was excited with HeNe laser (543nm) and measured using a long pass filter > 570nm. EGFP-$\alpha_1\beta_2\gamma_2$ largely corresponds to ER staining, whereas cytoplasmic fluorescence observed for EGFP-$\alpha_{1\text{-}mut}\beta_2\gamma_2$ could indicate degraded EGFP-$\alpha_{1\text{-}mut}$ proteins. $n > 50$. Scale bars = 10μm.

GABRA1 associated with CAE, which is the second mutation found in a human disease in this gene. Our functional, morphological, and biochemical experiments clearly show a complete loss of function of the mutant $GABA_A$ receptor. The results show that heteromeric $GABA_A$ receptors harboring the S326fs328X mutation are not integrated in the surface membrane and suggest that the mutant protein is probably degraded. These observations could well explain the occurrence of epileptic seizures by impairing GABAergic

synaptic inhibition, the most important inhibitory mechanism in the mammalian brain. The results are consistent with those obtained for mutations found in *GABRA1* and *GABRG2* in related epilepsy syndromes, which also lead to a loss of function of the $\alpha_1\beta_2\gamma_2$ receptor complex.[3–7,9,11–16] Thus, there is significant support for the hypothesis that the identified de novo mutation contributes to the cause of epilepsy in this "sporadic" case with IGE. Because we did not observe a dominant negative effect of the mutant on the WT receptor, the epileptic phenotype is supposed to occur due to a haploinsufficiency of the *GABRA1* gene. We expect more de novo mutations in other epilepsy-related genes to be found in the future, when sporadic cases will be systematically screened. However, the complex inheritance of IGE suggests that other, currently undetected genetic alterations also may contribute to CAE in the described single patient.

The overall frequency of mutations in any of the $GABA_A$ receptor subunit genes in humans is probably low. Altogether, only six mutations in *GABRA1* and *GABRG2* causing IGE with or without febrile seizures,[3–7] as well as two coding variants in *GABRD* possibly related to idiopathic epilepsy,[17] have been identified so far. Our study shows that mutations in *GABRA1* rarely occur in classic IGE, because only one mutation was found in 98 unrelated cases. More mutations might be detected when promotor and intronic regions would be analyzed.

Our observation expands the spectrum of epilepsy syndromes associated with *GABRA1* mutations and further supports the hypothesis that the classic IGE phenotypes share a common genetic background.[2,18,19] Because mutations in the gene *CLCN2,* encoding a chloride channel involved in neuronal chloride homeostasis, which is essential for GABAergic inhibition, can be also associated with a large spectrum of IGE phenotypes,[20] "inhibited inhibition" might emerge as a central mechanism in the pathophysiology of these common genetic epilepsy syndromes.

This work was supported by the Volkswagen-Stiftung (A.H., H.L.), the Bundesministerium für Bildung und Forschung (BMBF/NGFN2, A.H., H.L.), the Deutsche Forschungsgemeinschaft (DFG; Le1030/9-1, H.L.; Bu938/8-1, J.B., K.K.), a Heisenberg fellowship of the DFG (H.L.), and a travel grant from the Commission of European Affairs of the International League against Epilepsy for presenting preliminary results of this study at the 6th European Congress on Epileptology (S.M.).

We thank all patients and nonaffected family members for participating in the study. We also thank S. Bail, A. Bellan-Koch, and A. Riecker for expert technical assistance.

References

1. Mulley JC, Scheffer IE, Petrou S, Berkovic SF. Channelopathies as a genetic cause of epilepsy. Curr Opin Neurol 2003;16:171–176.
2. Lerche H, Weber YG, Jurkat-Rott K, Lehmann-Horn F. Ion channel defects in idiopathic epilepsies. Curr Pharm Des 2005; 11:2737–2752.
3. Baulac S, Huberfeld G, Gourfinkel-An I, et al. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the γ2-subunit gene. Nat Genet 2001;28:46–48.
4. Wallace RH, Marini C, Petrou S, et al. Mutant GABA(A) receptor γ2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 2001;28:49–52.
5. Harkin LA, Bowser DN, Dibbens LM, et al. Truncation of the GABA(A)-receptor γ2 subunit in a family with generalized epilepsy with febrile seizures plus. Am J Hum Genet 2002;70:530–536.
6. Kananura C, Haug K, Sander T, et al. A splice-site mutation in *GABRG2* associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 2002;59:1137–1141.
7. Cossette P, Liu L, Brisebois K, et al. Mutation of *GABRA1* in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet 2002;31:184–189.
8. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989;30:389–399.
9. Krampfl K, Maljevic S, Cossette P, et al. Molecular analysis of the A322D mutation in the α_1-subunit of the $GABA_A$ receptor causing juvenile myoclonic epilepsy. Eur J Neurosci 2005;22:10–20.
10. Kittler JT, Wang J, Connolly CN, et al. Analysis of $GABA_A$ receptor assembly in mammalian cell lines and hippocampal neurons using γ_2-subunit green fluorescent protein chimeras. Mol Cell Neurosci 2000;16:440–452.
11. Bowser DN, Wagner DA, Czajkowski C, et al. Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [γ2(R43Q)] found in human epilepsy. Proc Natl Acad Sci U S A 2002;99:15170–15175.
12. MacDonald RL, Bianchi MT, Feng H. Mutations linked to generalized epilepsy in humans reduce GABA(A) receptor current. Exp Neurol 2003;184:58–67.
13. Fisher JL. A mutation in the $GABA_A$ receptor α1 subunit linked to human epilepsy affects channel gating properties. Neuropharmacology 2004;46:629–637.
14. Gallagher MJ, Song L, Arain F, MacDonald RL. The juvenile myoclonic epilepsy GABA(A) receptor α1 subunit mutation A322D produces asymmetrical, subunit position-dependent reduction of heterozygous receptor currents and α1 subunit protein expression. J Neurosci 2004;24:5570–5578.
15. Kang J, MacDonald RL. The GABAA receptor γ2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of $\alpha1\beta2\gamma2S$ receptors in the endoplasmic reticulum. J Neurosci 2004;24:8672–8677.
16. Sancar F, Czajkowski C. A GABAA receptor mutation linked to human epilepsy (γ2R43Q) impairs cell surface expression of $\alpha\beta\gamma$ receptors. J Biol Chem 2004;279:47034–47039.
17. Dibbens LM, Feng HJ, Richards MC, et al. GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet 2004;13: 1315–1319.
18. Reutens DC, Berkovic SF. Idiopathic generalized epilepsy of adolescence: are the syndromes clinically distinct? Neurology 1995;45:1469–1476.
19. Marini C, Scheffer IE, Crossland KM, et al. Genetic architecture of idiopathic generalized epilepsy: clinical genetic analysis of 55 multiplex families. Epilepsia 2004;45:467–478.
20. Haug K, Warnstedt M, Alekov AK, et al. Mutations in the CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. Nat Genet 2003;33:527–532.

APPENDIX 3: SACRED DISEASE SECRETS REVEALED: THE GENETICS OF HUMAN EPILEPSY

Reference :

Turnbull J, Lohi H, Kearney JA, Rouleau GA, Delgado-Escueta AV, Meisler MH, Cossette P, and Minassian BA. Sacred disease secrets revealed: the genetics of human epilepsy. *Human Molecular Genetics* 2005; 14: 2491-2500.

Human Molecular Genetics, 2005, Vol. 14, No. 17 **2491–2500**
doi:10.1093/hmg/ddi250
Advance Access published on July 27, 2005

Sacred disease secrets revealed: the genetics of human epilepsy

Julie Turnbull[1,†], Hannes Lohi[1,†], Jennifer A. Kearney[2], Guy A. Rouleau[3], Antonio V. Delgado-Escueta[4], Miriam H. Meisler[2], Patrick Cossette[3] and Berge A. Minassian[1,*]

[1]Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8, [2]Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109-0618, USA, [3]Center for the Study of Brain Diseases, Centre Hospitalier de l'Université de Montréal, Notre Dame Hospital, Montreal, Quebec, Canada H2L 4M1 and [4]Epilepsy Center of Excellence, Greater Los Angeles VA Healthcare System, Los Angeles, CA 90073, USA

Received June 15, 2005; Accepted July 8, 2005

Neurons throughout the brain suddenly discharging synchronously and recurrently cause primarily generalized seizures. Discharges localized awhile in one part of the brain cause focal-onset seizures. A genetically determined generalized hyperexcitability had been predicted in primarily generalized seizures, but surprisingly the first epilepsy gene discovered, *CHRNA4*, was in a focal (frontal lobe)-onset syndrome. Another surprise with *CHRNA4* was its encoding of an ion channel present throughout the brain. The reason why *CHRNA4* causes focal-onset seizures is unknown. Recently, the second focal (temporal lobe)-onset epilepsy gene, *LGI1* (unknown function), was discovered. *CHRNA4* led the way to mutation identifications in 15 ion channel genes, most causing primarily generalized epilepsies. Potassium channel mutations cause benign familial neonatal convulsions. Sodium channel mutations cause generalized epilepsy with febrile seizures plus or, if more severe, severe myoclonic epilepsy of infancy. Chloride and calcium channel mutations are found in rare families with the common syndromes childhood absence epilepsy and juvenile myoclonic epilepsy (JME). Mutations in the *EFHC1* gene (unknown function) occur in other rare JME families, and yet in other families, associations are present between JME (or other generalized epilepsies) and single nucleotide polymorphisms in the *BRD2* gene (unknown function) and the malic enzyme 2 (*ME2*) gene. Hippocrates predicted the genetic nature of the 'sacred' disease. Genes underlying the 'malevolent' forces seizing 1% of humans have now been revealed. These, however, still account for a mere fraction of the genetic contribution to epilepsy. Exciting years are ahead, in which the genetics of this extremely common, and debilitating, neurological disorder will be solved.

I am about to discuss the disease called "sacred." It is not, in my opinion, any more divine or more sacred than any other diseases, but has a natural cause ... Its origin, like that of other diseases, lies in heredity ... The fact is that the cause of this affection is ... the brain ... My own view is that those who first attributed a sacred character to this malady were like the magicians, purifiers, charlatans, and quacks of our own day ... (1)

Hippocrates 470-410 BC

Two and a half millennia ago, the 'father of medicine' described epilepsy (1) and noted its genetic basis. The current decade marks the first unraveling of molecular alterations responsible for epilepsy and its heritability, which we review in this article.

The human brain is possibly the most complex structure in the universe (Fig. 1). The neuronal component consists of more than 20 billion cells, each connected with at least 10 000 others (2). Epilepsy is defined as a propensity to

*To whom correspondence should be addressed at: Program in Genetics and Genomic Biology and Department of Paediatrics (Neurology), The Hospital for Sick Children, Toronto, Canada M5G 1X8. Tel: +1 4168136291; Fax: +1 4168136334; Email: bminass@sickkids.ca
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

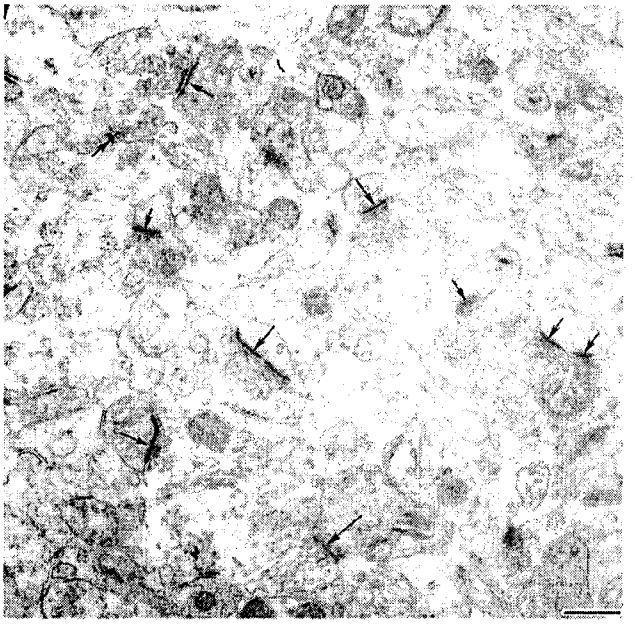


Figure 1. Electron micrograph of mouse neuropil. Several synapses are shown: black arrows point to the postsynaptic density, which is comprised of the neurotransmitter receptors; white arrows point to presynaptic vesicles, which contain the neurotransmitters. Bar = 500 nm. There are at least 200 quadrillion synapses in the human brain. Image courtesy of Dr Cameron Ackerley.

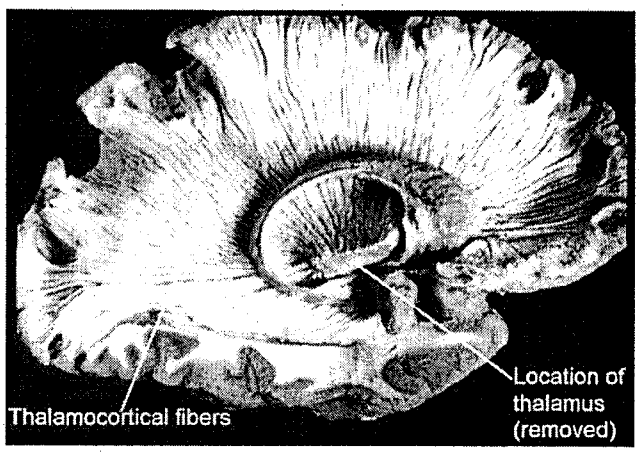


Figure 2. Thalamocortical fibers connect the thalamus to the entire cerebral cortex and allow it to synchronize cortical neuronal firing, in sleep and during generalized seizures.

seize; in practice, an individual who has two or more unprovoked seizures is epileptic and will usually continue to have seizures unless successfully treated. Epilepsy affects 1% of people worldwide, in an estimated 40% of whom it is genetically determined (3). Other causes of epilepsy include trauma, stroke and tumors.

In wakefulness, brain activity is in a state of apparent chaos. Like busy workers in a busy city, every neuron is acting and reacting, which in turn generating consciousness and the other characteristics and abilities of the mind. During sleep, specialized neurons in the thalamus with profuse connections to the entire brain (Fig. 2) gradually disrupt the individual activities of cortical neurons and entrain them all into monotonous rhythmic synchronized discharges (4). Therefore, synchronized activity of large numbers of neurons abolishes their normal 'wakeful' functions.

A seizure also consists of synchronized firing of large numbers of neurons. There are two main types of seizures. In primarily generalized seizures, the thalamocortical circuitry is involved early in the attack and results in synchronized firing of neurons brain-wide, unconsciousness and often violent rhythmic shaking of body parts. In focal-onset seizures, the synchronized activity is restricted to one part of the cortex (e.g. to the arm control area of the right hemisphere, resulting in left-arm shaking) and may or may not subsequently spread to recruit the thalamocortical pathways and result in secondary generalization (5).

Firing of an action potential by a neuron results from threshold depolarization of its cross-plasma membrane voltage. This voltage is regulated by numerous ion channels that open or close, some in response to synaptic neuromediators and others in response to changes in the voltage itself. It is easy to imagine that genetic mutations in singly or groups of channels will result in altered neuronal excitability, which, in certain situations, will cause recurrent firing and driving of a network of neurons into synchrony and a seizure. It is important to remember that 'increased excitability' is not synonymous with seizure, e.g. the increased excitability could be in inhibitory neurons. The final outcome of seizure due to excitability change depends very much on which neurons in which networks are affected.

This review restricts itself to the genetic causes of epilepsies occurring in previously well individuals with no evident underlying brain disorder before the onset of seizures. The first section addresses the roles of excitatory ion channels (Na and Ca), the second, inhibitory channels (K and Cl), and the third, non-ion channel genes. In all cases, so far the resultant epilepsies are autosomal dominant disorders or sporadic syndromes due to *de novo* hemizygous mutations (Table 1).

EXCITATORY ION CHANNEL EPILEPSY GENES

Sodium channel mutations

Nine genes encode α-subunits of voltage-gated Na channels. Four of these are highly expressed in neurons of the central nervous system: *SCN1A* ($Na_v1.1$), *SCN2A* ($Na_v1.2$), *SCN3A* ($Na_v1.3$) and *SCN8A* ($Na_v1.6$). The first evidence for a role of this gene family in epilepsy came from positional cloning of the inherited syndrome generalized epilepsy with febrile seizures plus (GEFS+), a mild disorder with a variable epileptic phenotype including fever-induced seizures. Two families with gene loci mapped to chromosome 2q24 families contained missense mutations in evolutionarily conserved residues of *SCN1A*, changing amino acid residues within or close to transmembrane segments of the protein (6). Since then, 13 additional missense mutations of *SCN1A* have been identified, accounting for ~10% of GEFS+ families tested (Fig. 3A). In functional assays, these missense mutations

Table 1. Epilepsy genes and corresponding syndromes

Genes	Neurological disorder	Chromosome	References
Na channel			
SCN2A	BFNIS (MIM 607745), GEFS+ (MIM 604233)	2q23–q24.3	(15,16)
SCN1B	GEFS+1 (MIM 604233)	19q13.1	(19)
SCN1A	GEFS+2 (MIM 604233), SMEI (607208)	2q24	(6,12)
Ca channel			
CACNB4	IGE (MIM 600669), JME (MIM 606904)	2q22–23	(28)
CACNA1A	EA2 (MIM 108500), FHM(MIM 141500), SCA6 (MIM 183086), IGE (MIM 600669)	19q	(31,33,89)
CACNA1H	CAE (MIM 607682)	16p13.3	(23)
ACh receptor			
CHRNA4	ADNFLE1 (MIM 6000513)	20q13.2–q13.3	(36)
CHRNB2	ADENFL3 (MIM 605375)	1p21	(90,91)
K channel			
KCNQ2	BFNC1 (MIM 125370), BFNC/myokymia (MIM 606437)	20q13.3	(92)
KCNQ3	BFNC2 (MIM 121201)	8q24	(43,44)
KCNA1	EA1 (MIM 160120), partial epilepsy	12p13	(45,93)
$GABA_A$ receptor			
GABRA1	JME (MIM 606904)	5q34	(60)
GABRG2	GEFS+3 (MIM 604233), CAE (MIM 607681)	5q34	(58,59)
GABRD	GEFS+ (MIM 604233)	1p36.3	(63)
Cl channel			
CLCN2	CAE (MIM 607682), EGMA (MIM 607628)	3q26	(66)
Non-ion channel			
LGI1	ADPEAF (MIM 600512)	10q24	(71,94)
EFHC1	JME (MIM 254770)	6p12–p11	(80)
BRD2	JME (MIM 254770)	6p21.3	(85)
ME2	IGE (MIM 600669)	18q21	(86)

BFNIS, benign familial neonatal and infantile seizures; IGE, idiopathic generalized epilepsy; EA, episodic ataxia; FHM, familial hemiplegic migraine; SCA, spinocerebellar ataxia; BFNC, benign familial neonatal convulsions; EGMA, epilepsy with grand mal upon awakening; ADPEAF, autosomal dominant partial epilepsy with auditory features.

produce subtle changes, such as increased persistent current and alterations in voltage-dependent gating, in biophysical parameters of channel activity (7–10). One GEFS+ mutation in the C-terminal cytoplasmic domain of *SCN1A* reduced interaction with the β1-subunit (11).

In addition to inherited mutations, it is now clear that *de novo* mutations of *SCN1A* account for ~50% of patients with severe myoclonic epilepsy of infancy (SMEI), a severe, early-onset epilepsy accompanied by intellectual deterioration (12). Nearly 200 independent mutations have been identified in affected children, and more than 90% of tested cases were sporadic. Approximately half of the SMEI mutations are nonsense mutations resulting in truncation of the channel protein and loss of channel activity (Fig. 3B). The observation that phenotypic severity is comparable to truncations close to the N-terminus of the protein and those close to the C-terminus indicates that loss of function is the common feature and demonstrates haploinsufficiency for *SCN1A* (13). Many missense mutations in SMEI patients also result in loss of function (14). It now appears that the *SCN1A*-related epilepsies comprise a spectrum of severity ranging from the mildest cases of GEFS+ characterized by childhood seizures without progression, through a wide range of variable phenotypes, to the devastating loss of function mutations in SMEI.

A small number of mutations have been identified in the closely related channel *SCN2A*, located 600 kb downstream from *SCN1A*. One missense mutation of *SCN2A* was found in a GEFS+ family (15), and six missense mutations were identified in patients with benign familial neonatal–infantile seizures, a mild syndrome that presents and remits in the first year of life (16,17) . One truncation mutation of *SCN2A* was identified in a patient with intractable epilepsy resembling SMEI (16,17).

The Na channel β-subunits, β1–β4, are small transmembrane proteins with an extracellular IgG loop (Fig. 3A). Association with the β-subunit influences α-subunit trafficking, stability and channel gating (18). Two different mutations in the β1 gene *SCN1B* have been identified in patients with GEFS+ (Fig. 3A) (19,20). The major effect of the β-subunit mutations seems to delay Na channel inactivation, similar to many of the *SCN1A* missense mutations in GEFS+ patients.

Calcium channel mutations

Ten genes encode α-subunits of voltage-gated Ca channels. Each α-subunit pairs with β- and α2δ-subunits (each of which has four subtypes), as well as a γ-subunit (eight subtypes) in certain instances. The magnitude of combinations linking these subunits allows for vast diversity in the regulation of Ca entry.

Several coding single nucleotide polymorphisms (SNPs) have been found in the *CACNA1H* gene in rare patients with

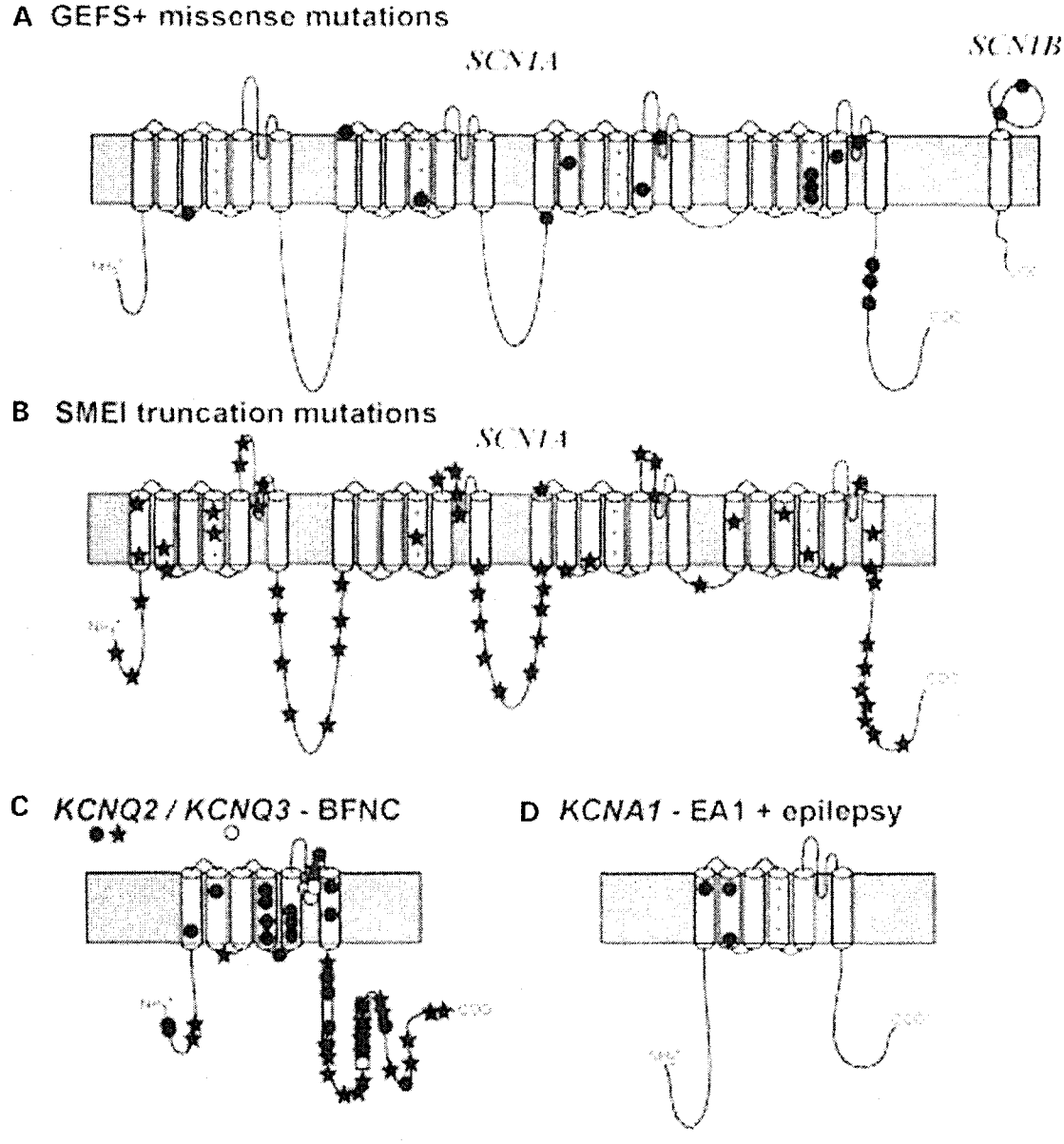


Figure 3. Epilepsy mutations in voltage-gated sodium and potassium channel genes. (**A**) Missense mutations of *SCN1A* and *SCN1B* in families with GEFS+. (**B**) *De novo* truncation mutations of *SCN1A* in patients with SMEI. (**C**) Mutations of *KCNQ2* (filled symbols) and *KCNQ3* (open symbols) in patients with benign familial neonatal convulsions. (**D**) Mutations of *KCNA1* in episodic ataxia 1 patients with seizures.

primarily generalized seizures, and not in a large number of controls. The patients included cases of childhood absence epilepsy (CAE) (21–23), a common pediatric epilepsy with frequent unconscious staring spells without convulsion. *CACNA1H* encodes an α-subunit that determines the $Ca_v3.2$ T-type calcium channel, which is critically linked to the synchronizing activity of the thalamus (4,24) and which is modulated by ethosuximide (25), the drug of choice for CAE. Furthermore, the various epilepsy-associated *CACNA1H* SNPs alter properties of the channel in ways predicted to generate seizures (23,26,27). Together, these results suggest that these SNPs are true mutations, explaining a small fraction of CAE.

A truncating mutation in *CACNB4* was found in a small family segregating juvenile myoclonic epilepsy (JME) (28), a very common epilepsy of adolescence with early morning jerks of the arms and generalized convulsions. The *CACNB4* observation has not yet been confirmed in other JME cases. Other *CACNB4* mutations, and mutations in *CACNA1A*, cause episodic ataxia type 2 and hemiplegic migraine. These disorders are not epilepsies, because they do not involve synchronized firings of large numbers of cortical neurons.

Nonetheless, many affected members in these families do also suffer bona fide seizures, indicating that these Ca channel genes also drive seizures when their properties are altered (28–33).

Mutations in the α4β2 nicotinic acetylcholine receptor

Nicotinic acetylcholine receptors are ligand (acetylcholine)-gated cation (Na and Ca) channels. They are pentamers of two types of subunits (α and β). α4 combined with β2 is the most common arrangement in brain (34). Six missense mutations causing an autosomal dominant nocturnal frontal lobe-onset epilepsy (ADNFLE) have been identified in α4β2, four in α4 (*CHRNA4*) and two in β2 (*CHRNB2*) (35). One of the α4 mutations is the first epilepsy-causing mutation discovered (36).

α4β2 occupies a particular neuronal location, and its mutations appear to cause epilepsy through a particularly interesting mechanism (37), which are worth elaborating (Fig. 4). It is present at the presynaptic side (axonal side) of both glutamatergic and GABAergic synapses. (Glutamate and GABA are the main excitatory and inhibitory neurotransmitters in brain. Glutamate receptors are Ca channels, which take in large amounts of Ca when stimulated. GABA receptors are Cl channels.) (38) The patch of presynaptic membrane occupied by α4β2 at these synapses is itself postsynaptic to a cholinergic synapse. Opening of α4β2 with cholinergic stimulation depends on a strong allosteric effect of extracellular Ca (39). When α4β2 opens, it adds local depolarization to the wave of depolarization arriving to the axon terminus with the action potential. ADNFLE mutations have in common the property of eliminating the allosteric Ca effect on α4β2 (37,40). This has led to the following theory to explain how these mutations cause sleep-induced seizures. Normally, with the recurrent stimulation of cortical neurons by the thalamus during sleep, Ca in glutamatergic synapses is reduced (absorbed by the glutamate receptors) and the Ca effect on α4β2 is diminished. At GABAergic synapses, Ca is not depleted and continues to activate α4β2. In sum, inhibitory GABAergic synapses are active and excitatory synapses are inactive during sleep. With ADNFLE mutations, Ca cannot activate α4β2, inhibition in brain is lost during a time of recurrent synchronizing firing by the thalamus and a seizure is generated (Fig. 4) (37).

INHIBITORY ION CHANNEL EPILEPSY GENES

Potassium channel mutations

The principal role of K channels is stabilization of the cell membrane potential including termination of intense activity, dampening of repetitive firing and lowering the effectiveness of excitatory inputs onto the cell. Among ion channels, K channel gene diversity is particularly striking, with 24 major classes and more than 80 different subunit genes (41). To date, three of these genes, *KCNQ2* (42,43), *KCNQ3* (44) and *KCNA1* (45,46), have been implicated in epilepsy.

KCNQ2 and KCNQ3 proteins combine in a heteromer to form the M type K current, which slowly activates in the voltage range of action potential initiation, repolarizing the membrane and suppressing repetitive firing (47). Mutations of *KCNQ2* and *KCNQ3* result in benign familial neonatal convulsions, where seizures occur essentially only in the first month of life and are inducible by provoked or natural arousal from sleep (48,49). Forty-eight mutations have been reported, most in *KCNQ2* (50–54) including three *de novo* mutations in non-inherited cases (55) (Fig. 3C).

Mutations in *KCNA1* were first identified in families with episodic ataxia type 1 (Fig. 3D). In two families, missense mutation in *KCNA1* was associated with focal-onset epilepsy(45,46). *KCNA1* is a rapidly activating, delayed-rectifier K channel ($K_v1.1$) that is primarily involved in the recovery phase of action potentials (41,56). Mutations in *KCNA1* associated with epilepsy dramatically reduce K currents *in vitro* (45,46), and knockout mice exhibit spontaneous focal-onset seizures (57).

Chloride channel mutations: the $GABA_A$ receptors

$GABA_A$ receptors are ligand (GABA)-gated Cl channels, which mediate fast inhibition. Their molecular structure comprises a heteropentameric protein complex assembled from 17 different classes of subunits (α1–6, β1–4, γ1–3, ...δ, ϵ, π and θ). Thus far, epilepsy-causing mutations have been identified in *GABRG2*, *GABRA1*, and *GABRD*, encoding, respectively, the α1, γ2 and δ-subunits (58–63), and *in vitro* functional studies have revealed that the majority of these mutations result in a reduction of GABA-activated Cl currents (Fig. 5) (58,60,63,64).

In *GABRG2*, two mutations cause GEFS+ (58,62) and two others result in febrile seizures and CAE (59,61). In *GABRA1*, one mutation segregates with rare JME families (60,65), and in *GABRD*, two missense mutations are associated with GEFS+ (63). One of these *GABRD* mutations, E177A, like *GABRG2* and *GABRA1* mutations, results in decreased amplitude of GABA-evoked currents, the other, R220C, does not. Whether it is a rare neutral variant or is associated with more subtle effects on the $GABA_A$ receptor remains to be determined. In the same study, another variant, R220H, was detected in a JME family, but it was also present in the general population with a frequency of 4.2%. Nonetheless, this polymorphism reduced GABA-evoked currents, which is expected to increase neuronal excitability. This and similar functional polymorphisms represent candidates for the modifier gene–dose effects anticipated in common epilepsies with complex inheritance, including JME and CAE.

Chloride channel mutations: the voltage-gated chloride channels

Five epilepsy mutations have been identified in the *CLCN2* gene (encoding the ClC-2 voltage-gated chloride channel) (66,67), three of which have been subject to functional studies. The M200fsX231 and del74–117 mutations completely abolish the ClC-2 current. In contrast, the G715E mutation appears to act through a different mechanism. It alters ClC-2 gating, resulting in an outward (reverse) chloride current expected to severely affect membrane potential stability and responses to polarity changes (66).

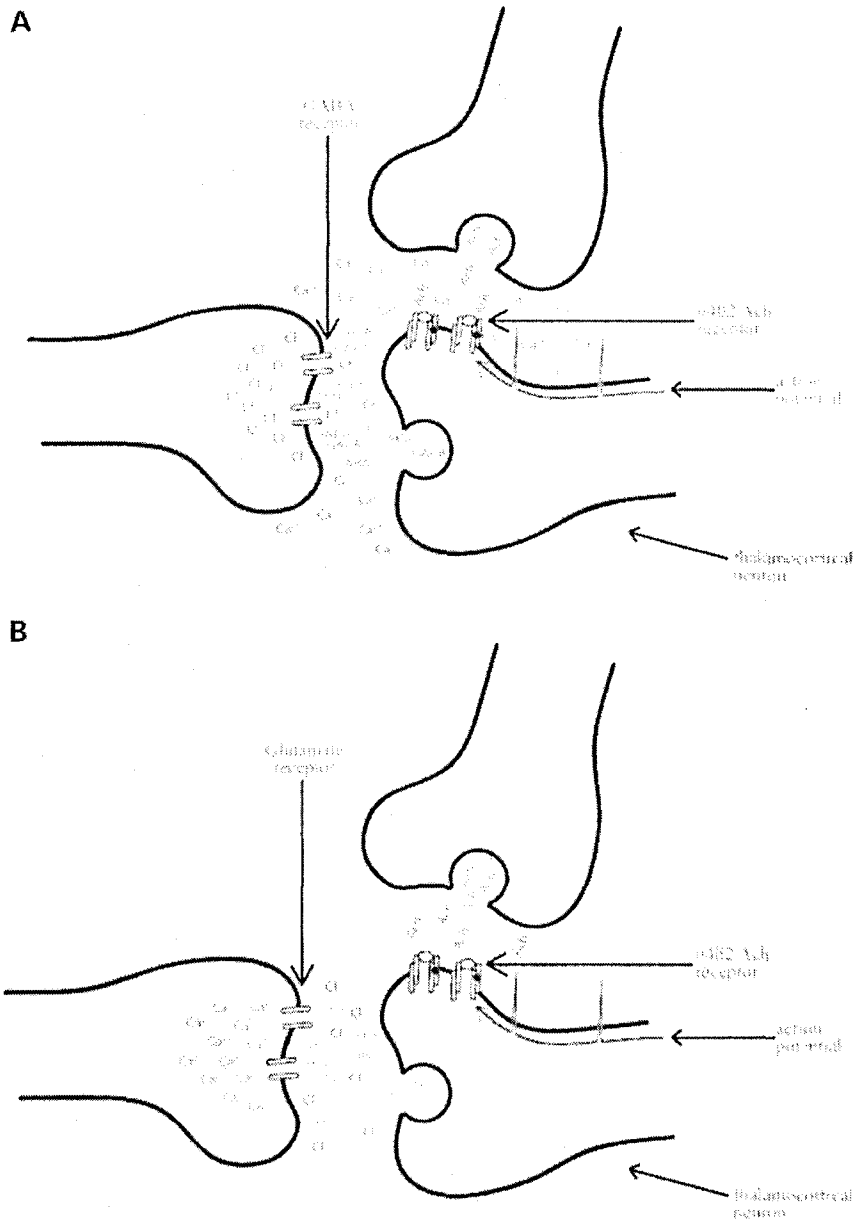


Figure 4. Model of sleep-related seizures due to mutations in the α4β2 nicotinic acetylcholine receptor (37,40). (**A**) At GABAergic synapses (inhibitory), Ca can no longer contribute to the activation of the acetylcholine receptor cation channel, because of mutation of its allosteric binding site on the channel (red dot). Decreased conduction through this cation channel results in decreased presynaptic amplification of the sleep-related trains of thalamocortical action potentials, and therefore, decreased synaptic transmission. (**B**) At glutamate synapses (excitatory), because synaptic Ca is quickly depleted into postsynaptic dendrites through glutamate receptors during repeated thalamocortical firing, it does not normally contribute to acetylcholine receptor activation. In sum, GABAergic synapses, but not glutamate synapses, are affected by the mutation during sleep, resulting in decreased inhibitory neurotransmission and seizure.

The phenotypic outcomes of the aforementioned mutations were remarkably varied. M200fsX231 was associated primarily with JME, del74–117 with generalized seizures upon awakening and G715E with juvenile absence epilepsy (a juvenile form of CAE) (66). These three epilepsies have primarily generalized seizures in common, but each has long been categorized as a separate clinical syndrome (65,68). Therefore, the *CLCN2* mutations in this study raise the possibility that variations in the same ion channel can underlie major syndrome-defining differences. Alternatively, *CLCN2* mutations merely predispose to generalized seizures, and modifier genes, different in each family, account for the phenotypic differences.

It is abundantly evident that epilepsy due to ion channel mutations is characterized by wide clinical and genetic heterogeneity. All the mutations reviewed earlier account for a mere

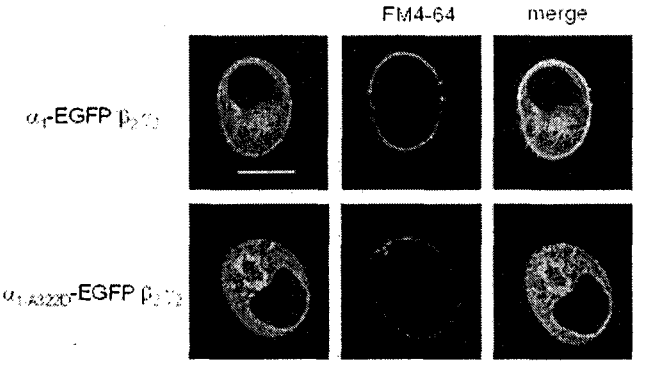


Figure 5. The A322D *GABRA1* mutation appears to reduce $GABA_A$ Cl currents through reduced surface expression of the mutated protein. HEK 293 cells were co-transfected with α1-EGFP/β2/γ2, α1-A322D-EGFP/β2/γ2, or EGFP alone and visualized using laser-scanning microscopy. Membrane specific labeling was performed by adding FM4-64 to the medium used for live confocal imaging. The overlay shows a clear localization in the surface membrane for wild-type, but not for mutant $GABA_A$ receptors (95). EGFP, enhanced green fluorescent protein.

fraction of the genetic contribution to epilepsy, and it is likely that many more ion channel mutations, singly or in groups, or mutations in proteins affecting ion channel functions will be found, tweaking the brain towards synchronized firings and seizures. However, epilepsy is also expected to result from miswirings in sections of the neural network, and perhaps some of the mutations discussed in the next section act in this fashion.

NON-ION CHANNEL EPILEPSY GENES

LGI1

Originally identified in glioma studies (69), the leucine-rich glioma-inactivated (*LGI1*) gene is currently considered not to play any important role in brain tumors (70). Instead, its mutations result in a focal-onset epilepsy with onset in or near the auditory center in the temporal lobe of the brain, resulting in auditory seizures with or without generalization to convulsion and unconsciousness (71–76).

Little is known about the *LGI1* protein function (named LGI1 and epitempin). It consists of an N-terminal leucine-rich repeat region and a C-terminal EAR (epilepsy-associated repeat) region (77), and it is a secreted protein (78,79). The EAR region is a common feature with the *Mass1* gene product mutated in the Frings mouse model of audiogenic epilepsy (77). Introduction of epilepsy-associated mutations results in unstable protein, suggesting that the mutations act through a loss of function mechanism (79). Finally, LGI1 appears to play a major role in suppressing the production of MMP1/3 through the phosphatidylinositol 3-kinase/ERK pathway. How LGI1 mutations result in seizure generation remains completely unknown, and why the temporal cortex is affected is equally mysterious. It is possible that LGI1 affects ion channels with particular relevance to auditory cortex or that it influences proper auditory cortex neuronal network establishment.

EFHC1

EFHC1 is yet another gene mutated in some families with JME. Its protein product, EFHC1 or myoclonin 1, localizes in the soma and dendrites of neurons in multiple brain regions. EFHC1 interacts with the R-type voltage-dependent Ca channel ($Ca_V2.3$) and leads to a specific increase of this current when expressed in tissue culture. Introduction of JME mutations greatly reduces the activating effect of EFHC1 on the channel (80).

EFHC1 may therefore cause JME through neuronal membrane electrical destabilization, as is the case in JME due to ion channel mutations. However, further studies raise an alternate or additional possibility. EFHC1 is pro-apoptotic, and the apoptosis it induces is reduced by *EFHC1* JME mutations. EFHC1-induced apoptosis is also specifically suppressed by a $Ca_V2.3$ antagonist, suggesting that it is driven by the Ca influx through this channel (80). During normal brain development, neuronal numbers and processes overshoot and are then trimmed as the final structure is established (81,82). The few JME brains that have been studied pathologically (83), or with detailed magnetic resonance imaging (84), reveal mildly thickened cerebral cortex and dystopic neurons. It is therefore possible that *EFHC1* mutations result in insufficient apoptotic shedding of unnecessary neurons during development and produce an imperfect, overpopulated and epileptogenic, cerebral network (80).

BRD2 and ME2

Finally, highly significant associations have been reported between non-coding SNPs in the *BRD2* gene and JME (85) and in the *ME2* gene and primarily generalized epilepsies including JME (86). BRD2 is a putative developmental transcription regulator expressed in brain and may be involved in the JME cortical microdysgenesis as mentioned earlier (85). *ME2* encodes malic enzyme 2, a mitochondrial enzyme involved in the synthesis of GABA, the ubiquitous inhibitory neuromediator (86).

The difficulty with the intriguing *BRD2* and *ME2* observations is in finding ways of establishing animal models to confirm the roles of these genes and as models for pathogenetic studies. This difficulty is shared with the increasing number of other common genetic diseases found segregating with SNPs. In most such instances, it is problematical to identify the effect of the SNP on its associated gene and find ways to replicate that effect in a mouse. Furthermore, most of these diseases are complex in inheritance, and one would need to identify and recreate several if not many participating polymorphisms. Perhaps, the solution will come not so much from engineered mice, but through detailed clinical and genetic studies in domesticated animals. A first canine epilepsy gene has already been discovered, albeit in the monogenic Lafora progressive myoclonus epilepsy (87). Epilepsy in dogs is five to 10 times more common than that in man (88). If, for example, naturally occurring JME could be characterized in dog, then one could attempt to replicate the genetic associations with *BRD2, ME2* and other JME genes in dog families, confirming the associations in a different organism and, at the same time, establishing an animal model.

Many more epilepsy genes than the ones reviewed in this article remain to be discovered. Epilepsy mutations affect proteins that regulate action potentials and synaptic function, both of which underlie neuronal communication. They also appear to affect proteins involved in proper cortical network establishment. Identifying epilepsy proteins and understanding their functions are clearly critical to better care for the tens of millions of patients afflicted with seizures (and with the devastating unpredictability of seizures). They are also of great value to the understanding of neuronal network formation and communication, i.e. ultimately, to the understanding of the human brain by the human brain.

Conflict of Interest statement. None declared.

REFERENCES

1. Riggs, A.J. and Riggs, J.E. (2005) Epilepsy's role in the historical differentiation of religion, magic, and science. *Epilepsia*, **46**, 452–453.
2. Mesulam, M.M. (1998) From sensation to cognition. *Brain*, **121** (Pt 6), 1013–1052.
3. Annegers, J.F., Rocca, W.A. and Hauser, W.A. (1996) Causes of epilepsy: contributions of the Rochester epidemiology project. *Mayo Clin. Proc.*, **71**, 570–575.
4. Steriade, M., McCormick, D.A. and Sejnowski, T.J. (1993) Thalamocortical oscillations in the sleeping and aroused brain. *Science*, **262**, 679–685.
5. Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P. and Engel, J., Jr. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*, **46**, 470–472.
6. Escayg, A., MacDonald, B.T., Meisler, M.H., Baulac, S., Huberfeld, G., An-Gourfinkel, I., Brice, A., LeGuern, E., Moulard, B., Chaigne, D. *et al.* (2000) Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat. Genet.*, **24**, 343–345.
7. Spampanato, J., Escayg, A., Meisler, M.H. and Goldin, A.L. (2001) Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. *J. Neurosci.*, **21**, 7481–7490.
8. Lossin, C., Wang, D.W., Rhodes, T.H., Vanoye, C.G. and George, A.L., Jr. (2002) Molecular basis of an inherited epilepsy. *Neuron*, **34**, 877–884.
9. Spampanato, J., Escayg, A., Meisler, M.H. and Goldin, A.L. (2003) Generalized epilepsy with febrile seizures plus type 2 mutation W1204R alters voltage-dependent gating of Na(v)1.1 sodium channels. *Neuroscience*, **116**, 37–48.
10. Lossin, C., Rhodes, T.H., Desai, R.R., Vanoye, C.G., Wang, D., Carniciu, S., Devinsky, O. and George, A.L., Jr. (2003) Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. *J. Neurosci.*, **23**, 11289–11295.
11. Spampanato, J., Kearney, J.A., de Haan, G., McEwen, D.P., Escayg, A., Aradi, I., MacDonald, B.T., Levin, S.I., Soltesz, I., Benna, P. *et al.* (2004) A novel epilepsy mutation in the sodium channel SCN1A identifies a cytoplasmic domain for beta subunit interaction. *J. Neurosci.*, **24**, 10022–10034.
12. Claes, L., Del-Favero, J., Ceulemans, B., Lagae, L., van Broeckhoven, C. and de Jonghe, P. (2001) *De novo* mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am. J. Hum. Genet.*, **68**, 1327–1332.
13. Meisler, M.H. and Kearney, J.A. (2005) Sodium channel mutations in epilepsy and other neurological disorders. *J. Clin. Invest.*, **115**, 2010–2017.
14. Rhodes, T.H., Lossin, C., Vanoye, C.G., Wang, D.W. and George, A.L., Jr. (2004) Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. *Proc. Natl Acad. Sci. USA*, **101**, 11147–11152.
15. Sugawara, T., Tsurubuchi, Y., Agarwala, K.L., Ito, M., Fukuma, G., Mazaki-Miyazaki, E., Nagafuji, H., Noda, M., Imoto, K., Wada, K. *et al.* (2001) A missense mutation of the Na+ channel alpha II subunit gene Na(v)1.2 in a patient with febrile and afebrile seizures causes channel dysfunction. *Proc. Natl Acad. Sci. USA*, **98**, 6384–6389.
16. Heron, S.E., Crossland, K.M., Andermann, E., Phillips, H.A., Hall, A.J., Bleasel, A., Shevell, M., Mercho, S., Seni, M.H., Guiot, M.C. *et al.* (2002) Sodium-channel defects in benign familial neonatal–infantile seizures. *Lancet*, **360**, 851–852.
17. Berkovic, S.F., Heron, S.E., Giordano, L., Marini, C., Guerrini, R., Kaplan, R.E., Gambardella, A., Steinlein, O.K., Grinton, B.E., Dean, J.T. *et al.* (2004) Benign familial neonatal–infantile seizures: characterization of a new sodium channelopathy. *Ann. Neurol.*, **55**, 550–557.
18. Isom, L.L. (2001) Sodium channel beta subunits: anything but auxiliary. *Neuroscientist*, **7**, 42–54.
19. Wallace, R.H., Wang, D.W., Singh, R., Scheffer, I.E., George, A.L., Jr, Phillips, H.A., Saar, K., Reis, A., Johnson, E.W., Sutherland, G.R. *et al.* (1998) Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. *Nat. Genet.*, **19**, 366–370.
20. Audenaert, D., Claes, L., Ceulemans, B., Lofgren, A., van Broeckhoven, C. and de Jonghe, P. (2003) A deletion in SCN1B is associated with febrile seizures and early-onset absence epilepsy. *Neurology*, **61**, 854–856.
21. Chen, Y., Lu, J., Pan, H., Zhang, Y., Wu, H., Xu, K., Liu, X., Jiang, Y., Bao, X., Yao, Z. *et al.* (2003) Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann. Neurol.*, **54**, 239–243.
22. Heron, S.E., Phillips, H.A., Mulley, J.C., Mazarib, A., Neufeld, M.Y., Berkovic, S.F. and Scheffer, I.E. (2004) Genetic variation of CACNA1H in idiopathic generalized epilepsy. *Ann. Neurol.*, **55**, 595–596.
23. Vitko, I., Chen, Y., Arias, J.M., Shen, Y., Wu, X.R. and Perez-Reyes, E. (2005) Functional characterization and neuronal modeling of the effects of childhood absence epilepsy variants of CACNA1H, a T-type calcium channel. *J. Neurosci.*, **25**, 4844–4855.
24. Suzuki, S. and Rogawski, M.A. (1989) T-type calcium channels mediate the transition between tonic and phasic firing in thalamic neurons. *Proc. Natl Acad. Sci. USA*, **86**, 7228–7232.
25. Coulter, D.A., Huguenard, J.R. and Prince, D.A. (1989) Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann. Neurol.*, **25**, 582–593.
26. Khosravani, H., Altier, C., Simms, B., Hamming, K.S., Snutch, T.P., Mezeyova, J., McRory, J.E. and Zamponi, G.W. (2004) Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. *J. Biol. Chem.*, **279**, 9681–9684.
27. Khosravani, H., Bladen, C., Parker, D.B., Snutch, T.P., McRory, J.E. and Zamponi, G.W. (2005) Effects of Ca(v)3.2 channel mutations linked to idiopathic generalized epilepsy. *Ann. Neurol.*, **57**, 745–749.
28. Escayg, A., de Waard, M., Lee, D.D., Bichet, D., Wolf, P., Mayer, T., Johnston, J., Baloh, R., Sander, T. and Meisler, M.H. (2000) Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am. J. Hum. Genet.*, **66**, 1531–1539.
29. Barrett, C.F., Cao, Y.Q. and Tsien, R.W. (2005) Gating deficiency in a familial hemiplegic migraine type 1 mutant P/Q-type calcium channel. *J. Biol. Chem.*, **280**, 24064–24071.
30. Jodice, C., Mantuano, E., Veneziano, L., Trettel, F., Sabbadini, G., Calandriello, L., Francia, A., Spadaro, M., Pierelli, F., Salvi, F. *et al.* (1997) Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum. Mol. Genet.*, **6**, 1973–1978.
31. Jouvenceau, A., Eunson, L.H., Spauschus, A., Ramesh, V., Zuberi, S.M., Kullmann, D.M. and Hanna, M.G. (2001) Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*, **358**, 801–807.
32. Holtmann, M., Opp, J., Tokarzewski, M. and Korn-Merker, E. (2002) Human epilepsy, episodic ataxia type 2, and migraine. *Lancet*, **359**, 170–171.
33. Chioza, B., Wilkie, H., Nashef, L., Blower, J., McCormick, D., Sham, P., Asherson, P. and Makoff, A.J. (2001) Association between the alpha(1a) calcium channel gene CACNA1A and idiopathic generalized epilepsy. *Neurology*, **56**, 1245–1246.
34. Dajas-Bailador, F. and Wonnacott, S. (2004) Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol. Sci.*, **25**, 317–324.
35. Combi, R., Dalpra, L., Tenchini, M.L. and Ferini-Strambi, L. (2004) Autosomal dominant nocturnal frontal lobe epilepsy—a critical overview. *J. Neurol.*, **251**, 923–934.

36. Steinlein, O.K., Mulley, J.C., Propping, P., Wallace, R.H., Phillips, H.A., Sutherland, G.R., Scheffer, I.E. and Berkovic, S.F. (1995) A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat. Genet.*, **11**, 201–203.
37. Rodrigues-Pinguet, N., Jia, L., Li, M., Figl, A., Klaassen, A., Truong, A., Lester, H.A. and Cohen, B.N. (2003) Five ADNFLE mutations reduce the Ca2+ dependence of the mammalian alpha4beta2 acetylcholine response. *J. Physiol.*, **550**, 11–26.
38. Wonnacott, S. (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci.*, **20**, 92–98.
39. Galzi, J.L., Bertrand, S., Corringer, P.J., Changeux, J.P. and Bertrand, D. (1996) Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor. *EMBO J.*, **15**, 5824–5832.
40. Rodrigues-Pinguet, N.O., Pinguet, T.J., Figl, A., Lester, H.A. and Cohen, B.N. (2005) Mutations linked to autosomal dominant nocturnal frontal lobe epilepsy affect allosteric ca2+ activation of the {alpha}4{beta}2 nicotinic acetylcholine receptor. *Mol. Pharmacol.*, **68**, 487–501.
41. Hille, B. (2001) *Ion Channels of Excitable Membranes*, 3rd edn. Sinauer, Sunderland, MA.
42. Biervert, C., Schroeder, B.C., Kubisch, C., Berkovic, S.F., Propping, P., Jentsch, T.J. and Steinlein, O.K. (1998) A potassium channel mutation in neonatal human epilepsy. *Science*, **279**, 403–406.
43. Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V. *et al.* (1998) A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat. Genet.*, **18**, 25–29.
44. Charlier, C., Singh, N.A., Ryan, S.G., Lewis, T.B., Reus, B.E., Leach, R.J. and Leppert, M. (1998) A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat. Genet.*, **18**, 53–55.
45. Zuberi, S.M., Eunson, L.H., Spauschus, A., de Silva, R., Tolmie, J., Wood, N.W., McWilliam, R.C., Stephenson, J.P., Kullmann, D.M. and Hanna, M.G. (1999) A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*, **122** (Pt 5), 817–825.
46. Eunson, L.H., Rea, R., Zuberi, S.M., Youroukos, S., Panayiotopoulos, C.P., Liguori, R., Avoni, P., McWilliam, R.C., Stephenson, J.B., Hanna, M.G. *et al.* (2000) Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. *Ann. Neurol.*, **48**, 647–656.
47. Rogawski, M.A. (2000) KCNQ2/KCNQ3 K+ channels and the molecular pathogenesis of epilepsy: implications for therapy. *Trends Neurosci.*, **23**, 393–398.
48. Rett, A. and Teubel, R. (1964) Neugeborenenkrämpfe im Rhamen einer epileptisch belasten Familie. *Wien. Klein. Wochenschr.*, **76**, 609–612.
49. Plouin, P. and Anderson, V.E. (2002) *Epileptic Syndromes in Infancy, Childhood and Adolescence*. John Libbey, London.
50. Richards, M.C., Heron, S.E., Spendlove, H.E., Scheffer, I.E., Grinton, B., Berkovic, S.F., Mulley, J.C. and Davy, A. (2004) Novel mutations in the KCNQ2 gene link epilepsy to a dysfunction of the KCNQ2-calmodulin interaction. *J. Med. Genet.*, **41**, e35.
51. Singh, N.A., Westenskow, P., Charlier, C., Pappas, C., Leslie, J., Dillon, J., Anderson, V.E., Sanguinetti, M.C. and Leppert, M.F. (2003) KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain*, **126**, 2726–2737.
52. Borgatti, R., Zucca, C., Cavallini, A., Ferrario, M., Panzeri, C., Castaldo, P., Soldovieri, M.V., Baschirotto, C., Bresolin, N., Dalla Bernardina, B. *et al.* (2004) A novel mutation in KCNQ2 associated with BFNC, drug resistant epilepsy, and mental retardation. *Neurology*, **63**, 57–65.
53. Tang, B., Li, H., Xia, K., Jiang, H., Pan, Q., Shen, L., Long, Z., Zhao, G. and Cai, F. (2004) A novel mutation in KCNQ2 gene causes benign familial neonatal convulsions in a Chinese family. *J. Neurol. Sci.*, **221**, 31–34.
54. Pereira, S., Roll, P., Krizova, J., Genton, P., Brazdil, M., Kuba, R., Cau, P., Rektor, I. and Szepetowski, P. (2004) Complete loss of the cytoplasmic carboxyl terminus of the KCNQ2 potassium channel: a novel mutation in a large Czech pedigree with benign neonatal convulsions or other epileptic phenotypes. *Epilepsia*, **45**, 384–390.
55. Claes, L.R., Ceulemans, B., Audenaert, D., Deprez, L., Jansen, A., Hasaerts, D., Weckx, S., Claeys, K.G., Del-Favero, J., van Broeckhoven, C. *et al.* (2004) *De novo* KCNQ2 mutations in patients with benign neonatal seizures. *Neurology*, **63**, 2155–2158.
56. Meisler, M.H., Kearney, J., Ottman, R. and Escayg, A. (2001) Identification of epilepsy genes in human and mouse. *Annu. Rev. Genet.*, **35**, 567–588.
57. Smart, S.L., Lopantsev, V., Zhang, C.L., Robbins, C.A., Wang, H., Chiu, S.Y., Schwartzkroin, P.A., Messing, A. and Tempel, B.L. (1998) Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron*, **20**, 809–819.
58. Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'homme, J.F., Baulac, M., Brice, A., Bruzzone, R. and LeGuern, E. (2001) First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat. Genet.*, **28**, 46–48.
59. Wallace, R.H., Marini, C., Petrou, S., Harkin, L.A., Bowser, D.N., Panchal, R.G., Williams, D.A., Sutherland, G.R., Mulley, J.C., Scheffer, I.E. *et al.* (2001) Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat. Genet.*, **28**, 49–52.
60. Cossette, P., Liu, L., Brisebois, K., Dong, H., Lortie, A., Vanasse, M., Saint-Hilaire, J.M., Carmant, L., Verner, A., Lu, W.Y. *et al.* (2002) Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat. Genet.*, **31**, 184–189.
61. Kananura, C., Haug, K., Sander, T., Runge, U., Gu, W., Hallmann, K., Rebstock, J., Heils, A. and Steinlein, O.K. (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch. Neurol.*, **59**, 1137–1141.
62. Harkin, L.A., Bowser, D.N., Dibbens, L.M., Singh, R., Phillips, F., Wallace, R.H., Richards, M.C., Williams, D.A., Mulley, J.C., Berkovic, S.F. *et al.* (2002) Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am. J. Hum. Genet.*, **70**, 530–536.
63. Dibbens, L.M., Feng, H.J., Richards, M.C., Harkin, L.A., Hodgson, B.L., Scott, D., Jenkins, M., Petrou, S., Sutherland, G.R., Scheffer, I.E. *et al.* (2004) GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. *Hum. Mol. Genet.*, **13**, 1315–1319.
64. McDonald, A.J., Mascagni, F. and Muller, J.F. (2004) Immunocytochemical localization of GABABR1 receptor subunits in the basolateral amygdala. *Brain Res.*, **1018**, 147–158.
65. (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia*, **30**, 389–399.
66. Haug, K., Warnstedt, M., Alekov, A.K., Sander, T., Ramirez, A., Poser, B., Maljevic, S., Hebeisen, S., Kubisch, C., Rebstock, J. *et al.* (2003) Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nat. Genet.*, **33**, 527–532.
67. D'Agostino, D., Bertelli, M., Gallo, S., Cecchin, S., Albiero, E., Garofalo, P.G., Gambardella, A., St Hilaire, J.M., Kwiecinski, H., Andermann, E. *et al.* (2004) Mutations and polymorphisms of the CLCN2 gene in idiopathic epilepsy. *Neurology*, **63**, 1500–1502.
68. Roger, J., Bureau, M., Dravet, C., Genton, P., Tassinari, C.A. and Wolf, P. (2002) *Epileptic Syndromes in Infancy, Childhood and Adolescence*. John Libbey, London.
69. Chernova, O.B., Somerville, R.P. and Cowell, J.K. (1998) A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene*, **17**, 2873–2881.
70. Gu, W., Brodtkorb, E., Piepoli, T., Finocchiaro, G. and Steinlein, O.K. (2005) LGI1: a gene involved in epileptogenesis and glioma progression? *Neurogenetics*, **6**, 59–66.
71. Kalachikov, S., Evgrafov, O., Ross, B., Winawer, M., Barker-Cummings, C., Martinelli Boneschi, F., Choi, C., Morozov, P., Das, K., Teplitskaya, E. *et al.* (2002) Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat. Genet.*, **30**, 335–341.
72. Morante-Redolat, J.M., Gorostidi-Pagola, A., Piquer-Sirerol, S., Saenz, A., Poza, J.J., Galan, J., Gesk, S., Sarafidou, T., Mautner, V.F., Binelli, S. *et al.* (2002) Mutations in the LGI1/epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum. Mol. Genet.*, **11**, 1119–1128.
73. Pizzuti, A., Flex, E., Di Bonaventura, C., Dottorini, T., Egeo, G., Manfredi, M., Dallapiccola, B. and Giallonardo, A.T. (2003) Epilepsy with auditory features: a LGI1 gene mutation suggests a loss-of-function mechanism. *Ann. Neurol.*, **53**, 396–399.

74. Michelucci, R., Poza, J.J., Sofia, V., de Feo, M.R., Binelli, S., Bisulli, F., Scudellaro, E., Simionati, B., Zimbello, R., D'Orsi, G. *et al.* (2003) Autosomal dominant lateral temporal epilepsy: clinical spectrum, new epitempin mutations, and genetic heterogeneity in seven European families. *Epilepsia*, **44**, 1289–1297.
75. Hedera, P., Abou-Khalil, B., Crunk, A.E., Taylor, K.A., Haines, J.L. and Sutcliffe, J.S. (2004) Autosomal dominant lateral temporal epilepsy: two families with novel mutations in the LGI1 gene. *Epilepsia*, **45**, 218–222.
76. Ottman, R., Winawer, M.R., Kalachikov, S., Barker-Cummings, C., Gilliam, T.C., Pedley, T.A. and Hauser, W.A. (2004) LGI1 mutations in autosomal dominant partial epilepsy with auditory features. *Neurology*, **62**, 1120–1126.
77. Scheel, H., Tomiuk, S. and Hofmann, K. (2002) A common protein interaction domain links two recently identified epilepsy genes. *Hum. Mol. Genet.*, **11**, 1757–1762.
78. Staub, E., Perez-Tur, J., Siebert, R., Nobile, C., Moschonas, N.K., Deloukas, P. and Hinzmann, B. (2002) The novel EPTP repeat defines a superfamily of proteins implicated in epileptic disorders. *Trends Biochem. Sci.*, **27**, 441–444.
79. Senechal, K.R., Thaller, C. and Noebels, J.L. (2005) ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. *Hum. Mol. Genet.*
80. Suzuki, T., Delgado-Escueta, A.V., Aguan, K., Alonso, M.E., Shi, J., Hara, Y., Nishida, M., Numata, T., Medina, M.T., Takeuchi, T. *et al.* (2004) Mutations in EFHC1 cause juvenile myoclonic epilepsy. *Nat. Genet.*, **36**, 842–849.
81. Hua, J.Y., Smear, M.C., Baier, H. and Smith, S.J. (2005) Regulation of axon growth *in vivo* by activity-based competition. *Nature*, **434**, 1022–1026.
82. Ottersen, O.P. (2005) Neurobiology: sculpted by competition. *Nature*, **434**, 969.
83. Meencke, H.J. and Veith, G. (1999) *The Relevance of Slight Migrational Disturbances (Microdysgenesis) to the Etiology of the Epilepsies.* Lippincott Williams and Wilkins, Philadelphia.
84. Woermann, F.G., Sisodiya, S.M., Free, S.L. and Duncan, J.S. (1998) Quantitative MRI in patients with idiopathic generalized epilepsy. Evidence of widespread cerebral structural changes. *Brain*, **121** (Pt 9), 1661–1667.
85. Pal, D.K., Evgrafov, O.V., Tabares, P., Zhang, F., Durner, M. and Greenberg, D.A. (2003) BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am. J. Hum. Genet.*, **73**, 261–270.
86. Greenberg, D.A., Cayanis, E., Strug, L., Marathe, S., Durner, M., Pal, D.K., Alvin, G.B., Klotz, I., Dicker, E., Shinnar, S. *et al.* (2005) Malic enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. *Am. J. Hum. Genet.*, **76**, 139–146.
87. Lohi, H., Young, E.J., Fitzmaurice, S.N., Rusbridge, C., Chan, E.M., Vervoort, M., Turnbull, J., Zhao, X.C., Ianzano, L., Paterson, A.D. *et al.* (2005) Expanded repeat in canine epilepsy. *Science*, **307**, 81.
88. Licht, B.G., Licht, M.H., Harper, K.M., Lin, S., Curtin, J.J., Hyson, L.L. and Willard, K. (2002) Clinical presentations of naturally occurring canine seizures: similarities to human seizures. *Epilepsy Behav.*, **3**, 460–470.
89. Ophoff, R.A., Terwindt, G.M., Vergouwe, M.N., van Eijk, R., Oefner, P.J., Hoffman, S.M., Lamerdin, J.E., Mohrenweiser, H.W., Bulman, D.E., Ferrari, M. *et al.* (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell*, **87**, 543–552.
90. de Fusco, M., Becchetti, A., Patrignani, A., Annesi, G., Gambardella, A., Quattrone, A., Ballabio, A., Wanke, E. and Casari, G. (2000) The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat. Genet.*, **26**, 275–276.
91. Phillips, H.A., Favre, I., Kirkpatrick, M., Zuberi, S.M., Goudie, D., Heron, S.E., Scheffer, I.E., Sutherland, G.R., Berkovic, S.F., Bertrand, D. *et al.* (2001) CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. *Am. J. Hum. Genet.*, **68**, 225–231.
92. Dedek, K., Kunath, B., Kananura, C., Reuner, U., Jentsch, T.J. and Steinlein, O.K. (2001) Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K+ channel. *Proc. Natl Acad. Sci. USA*, **98**, 12272–12277.
93. Browne, D.L., Gancher, S.T., Nutt, J.G., Brunt, E.R., Smith, E.A., Kramer, P. and Litt, M. (1994) Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat. Genet.*, **8**, 136–140.
94. Gu, W., Brodtkorb, E. and Steinlein, O.K. (2002) LGI1 is mutated in familial temporal lobe epilepsy characterized by aphasic seizures. *Ann. Neurol.*, **52**, 364–367.
95. Krampfl, K., Maljevic, S., Cossette, P., Ziegler, E., Rouleav, G.A. Lerche, H., Bufler, J. (2005) Molecular analysis of the A322D mutation in the GABA receptor alpha-subnit causing juvenile myoclonic epilepsy. *Eur. J. Neurosci.*, **22**, 10–20.

APPENDIX 4: HUMAN ETHICS APPROVAL CERTIFICATE